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THYROID GLAND DEFICIENCY IN CHRONIC ARTHRITIS*

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THE complexity of the problems of chronic arthritis is apparent to all, particularly to those who are students of the disease. The American Committee for the Control of Rheumatism defines arthritis as a "generalized disease with joint manifestations." It may involve cartilage, bone, tendon, muscle, and blood vessel, as well as synovial surfaces. It would seem evident that any factor inside or outside the body which may injure directly or indirectly the integrity of a joint must be looked upon as a causal agent. The most successful treatment of the disease is that which seeks to determine the entire etiology in each case, to remove or arrest as many of these factors as may be, and to return the joints, and the patient, to the greatest possible degree of normal function. Such a problem requires the closest cooperation of internists and orthopedic surgeons.

Infection and diet have received much attention in recent years as important etiologic factors. Thyroid deficiency has also been suggested as another, and, while there are many references to it in the literature, its rôle has not been clearly defined. In this paper, we are attempting to show that it occurs quite frequently in patients with arthritis, especially in arthritis of the hypertrophic type, and that its correction leads to improvement in the joint condition.

REVIEW OF THE LITERATURE

Endocrine dysfunction has long been suggested as one cause of the greater incidence (3:1) of chronic arthritis among women as compared to men. It is generally recognized that the onset of the disease is often near the menopause, and some writers[1, 2, 3] maintain that menopausal arthritis is more or less distinct from the ordinary hypertrophic (osteoarthritic) or atrophic (rheumatoid) types, whether of infectious or metabolic etiology. There is no agreement as to the gland which is most at fault. The thyroid is incriminated by some, the ovary and pituitary

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by others, and the whole group by still others. Crowe,[1] however, feels that glandular deficiency is not necessary to explain these age and sex differences.

The association of arthritis with myxedema and hypothyroidism has attracted the attention of a few students for many years. Llewellyn[4] reports that the incidence of osteoarthritis seems to be abnormally high in regions in England where goiter is endemic, and he quotes McCarrison as saying that in goitrous regions in the Himalaya Mountains the coexistence in the same subject of goiter and arthritis is so common as to suggest a similarity of origin. He recites cases of his own, and others from the medical literature, in which arthritis followed closely upon hyperthyroidism which had been treated by x-rays, surgical removal or functional exhaustion of the gland. Mutch[5] in a series of 200 cases of chronic arthritis found that "minor forms of subthyroidism" were very common, while well defined myxedema or goiter of considerable size were seen in 14 per cent of the cases.

Jones[6] writes, "I have frequently observed in women at the menopause, the subjects of osteoarthritis of the knees, a peculiar change in the consistency of the skin and subcutaneous tissues, more particularly of the lower limbs. The overlying skin is usually harsh, dry and not infrequently scaly, has lost its suppleness and is with difficulty lifted up from the subjacent tissues, which to palpation feel tense, elastic, and, like the skin itself, much thickened. No pitting is to be observed on pressure, and the physical signs appear independently of renal and cardiac complications. The administration of thyroid substance in some instances was quickly followed by a diminution in swelling when present, and also in restoring a more natural consistency to the affected tissues.

"Whether the infiltration of the skin and subcutaneous tissues is identical with the diffuse, brawny infiltration noted occasionally by Garrod in the fingers of patients affected with multiple osteoarthritis of the hands, I am unable to say. As to its nature, one can only speculate; but objectively, at all events, it appears indistinguishable from the solid swelling associated with myxedema. Its localized distribution forbids our identifying it with this affection, but, at the same time, we must remember that physicians following Hertoghe are learning to postulate the existence of states intermediate between avowed myxedema and the normal 'formes frustees,' so to speak, analogous to the larval forms of Graves' disease, and presumably due to variable degrees of thyroid inadequacy."

Other writers confirm Jones as to the frequency of the symptoms of hypothyroidism in arthritic patients. Thus, Emerson[7] comments on the ease of fatigue, paresthesias, parchment-like skin, and dry, rough and brittle finger nails. Coates[1] emphasizes the loss of hair, increased weight of the body, dryness of the skin, inability to sweat, scanty or absent menstrual periods and increased sensibility to cold.

McCarrison[8] states that in patients with thyroid gland deficiency "The cartilages are also infiltrated and the joints are painful, stiff and may crackle. 'If the hand is applied to the joint (knee) when flexed, there will be a sensation like the crackling of frosted snow. This is a very characteristic sign; indeed, it is almost pathognomonic.' (Hertoghe) 'Rheumatic' pains and soreness in the bones and joints are a frequent source of complaint, and true rheumatoid arthritis is not uncommonly associated with the disease. Hydrarthrosis may occur (Dalche)."

Cecil and Archer[3] also reported crepitation in the knees with little limitation in motion, as well as excess fat about the hips and breast, round shoulders, lordosis, ptosis and flat feet.

Foster[9] writing about myxedema, says "In adults there is often soreness of the joints and relaxation of the ligaments, leading to flat foot and knock knee. A peculiar grating of the joints on motion has been described. . . . . Pains in the back and shoulders are common, and women complain of pain in their arms when doing up their hair; muscular cramps are usual. The muscles, as a rule, fatigue very readily . . . . When standing, the myxedematous patient 'droops all over,' the shoulders fall forward, the head hangs and the body is not erect. Ptosis of the viscera has been attributed to the relaxed abdominal wall."

In their study of 45 cases of hypothyroidism at the Massachusetts General Hospital, Means and Krantz[10] found that all complained of a feeling of fatigue or weakness and of pale and dry skin; 90 per cent had edema of the eyelids and face, increased weight, sensitivity to cold and absence of sweating; 80 per cent had numbness and tingling and loss of hair, and about 70 per cent had brittle nails; 18.9 per cent had symptoms and signs referable to the elbow joints, 56.4 per cent to the knees, and 34.2 per cent to other joints.

Studies of the basal metabolic rate in patients with chronic arthritis have given confusing results. Pemberton and Tompkins[11] found that 80 per cent of their 29 cases fell within normal limits (plus and minus 10 per cent) and that 20 per cent were slightly low (minus 11 per cent to minus 21 per cent). The low rates seemed to bear no relation to the severity of the disease, age or condition of the patient, atrophy of muscles, edema, or other factors. Cecil, Barr and DuBois[12] studied 4 cases of chronic arthritis in the calorimeter and concluded that they had no disturbance of metabolism. Holmes[13] reported normal readings in his 50 cases.

Boothby and Sandiford[26, 27] found basal metabolic readings between minus 15 and plus 15 in 93 per cent of 69 arthritic patients. The rate was below minus 15 in 4 of these patients. Hench[28] found that 80 per cent of 115 arthritic patients had basal metabolic determinations between plus 10 and minus 10, about equally divided on the minus and plus sides. Five slightly higher rates were obtained in one case of thyroiditis and in four cases of thyroid adenomas. In the infectious group (atrophic) six rates were between minus 10 and minus 20. There was a slight tendency for lower normal rates in the senile (hypertrophic) group. He quotes DuBois as having stated that in chronic infectious arthritis it would seem that the change in basal metabolism is such as might be expected in a crippling disease where the patients are forced to lead sedentary lives.

Swaim,[29] however, found that of his series of 312 cases, only 61 per cent fell between the normal limits of plus and minus 10, while 14 per cent were slightly increased (plus 10 to plus 15) and 25 per cent were decreased (minus 10 to minus 30). He found no evidence that hyperthyroidism and hypothyroidism are characteristically associated with arthritis. He believed that "abnormal metabolism with a tendency to a minus rate is characteristic of arthritis, especially in the early years, having a tendency to return to normal as the duration of the disease lengthens."

Swaim and Spear[14] agreed with Pemberton that the age of the individual and the duration of the activity of the disease had no apparent effect on the basal metabolic rate. They found that in the chronic infectious type of case there was a tendency toward an increased rate and in the hypertrophic case to a decreased rate.

Brown[15] found that of 25 cases of hypertrophic arthritis in which careful basal metabolic readings were made, 16 gave readings less than minus 10, and of these 10 were less than minus 15 and 5 less than minus 20.

Thompson[16] believes that an endocrine disturbance is one of the etiologic factors in arthritis, but believes no single gland to be responsible. We might quote many other writers who believe thyroid or other glandular deficiency to play a part in the etiology of chronic arthritis.

The effect of the administration of thyroid substance to patients with chronic arthritis likewise is in dispute. Jones,[6] Thompson,[16] Mutch,[5] Swaim,[14, 29] Brown[15] and Cecil,[17] among others, advocate its use. Pemberton[11] and other writers are in doubt. Swaim says that it "does not raise the rate in four-fifths of the cases, but if it is carefully given, beneficial results are obtained in spite of the fact that in doses which can be tolerated it may not change the metabolic rate. The chief improvement is in circulation, muscle tone, weight and vitality."

PRESENTATION OF DATA

In view of the indeterminate nature of the situation, it seemed of value to contribute an analysis of our experience. For this purpose, we have studied the records of 300 patients with chronic nontuberculous arthritis for the symptoms and signs of thyroid deficiency, the basal metabolic rates, and the effect of thyroid gland therapy when indicated. No selection of cases was made. The records of 100 private patients were taken at random from the files of the last ten years, and, in the same manner, those of 200 patients seen in the wards and arthritic clinic of the Out-Door Department of this Hospital in the last seven years. This method had the disadvantage of including some cases which we had not seen, cases with short observation periods and possibly incomplete data from the standpoint of moderate endocrine disturbances. It had the advantage of

representing a true cross section of the sort of arthritic patient we have been dealing with during this period, and of avoiding overstatement.

It happened that by chance 150 of the cases fell into the group of atrophic (rheumatoid) arthritis (106 of the hospital and clinic cases and 44 of the private) and an equal number into that of hypertrophic (osteoarthritic) arthritis (94 of the hospital cases and 56 of the private). To avoid confusion, no further subdivisions were made. The diagnosis in each instance was arrived at through the gross appearance of the joints and the roentgenographic findings. This was often a difficult matter. Some of the hypertrophic patients seemed to react like those with atrophic changes. Many of those who had had atrophic arthritis for a long time had acquired hypertrophic elements through mechanical irritation and efforts at repair. When a case was found to be classified as a mixed type, it was placed arbitrarily in the group to which it most nearly corresponded.

This group of patients does not represent a cross section of all types of arthritis. Cases clearly infectious in origin, such as gonorrheal arthritis, pneumococcal arthritis, or arthritis in association with rheumatic fever, did not usually reach our clinic, and were eliminated from the cases chosen from the files. Those patients whose arthritis was not obviously related to infection, or who had failed to improve with the removal of infection and with other therapy, were the patients generally sent to us. They rarely had fever, leucocytosis, or hot joints. Readers who see a larger proportion of cases of these types should, therefore, have in mind the character of this group. However, we believe some of these patients, especially those with atrophic arthritis, harbored an infectious process which played an important etiologic rôle.

Female patients outnumbered the males, as always, in the ratio of about 3 to 1. There were 106 females in the atrophic group and 112 in the hypertrophic; 44 males were classed as atrophic and 38 as hypertrophic. The average age of those with atrophic arthritis was 45, and of those with hypertrophic arthritis 54. That this disparity is not as great as usual is due to the fact that many of the patients came to us years after the beginning of their disease.

TABLE I

THE INCIDENCE OF SYMPTOMS WHICH MIGHT BE ATTRIBUTABLE TO THYROID DEFICIENCY IN 300 CASES OF CHRONIC ARTHRITIS

		ATROPHIC ARTHRITIS 150 CASES		HYPERTROPHIC ARTHRITIS 150 CASES	
		NO.	%	NO.	%
1	Sensitivity to cold	64	42.6	66	44
2	Cold hands and feet	73	48.6	76	50
3	Dry skin	59	39.3	94	62.6
4	Ease of tire	135	90	141	94
5	Symptoms out of proportion to physical findings	24	16	38	25.3
6	Arthralgia	24	16	40	26.6
7	Numbness	38	25.3	73	49
8	Brittle nails	38	25.3	66	44
9	Falling hair	23	15.3	59	39.3
10	Edema about the eyes	36	24	58	38.6
11	Obesity	53	35.3	99	66

Table I summarizes the frequency with which occurred the symptoms which might be caused by thyroid deficiency in these patients. Only the positive findings were recorded; where the symptoms were not mentioned, as often happened, they were considered to be absent. Their incidence tended to be greater in the private cases, since they were closely observed in this regard, but the figures for the hospital ward and the clinic cases show the same differences between the two types of arthritis. Patients of both types were equally sensitive to cold (42 per cent of the atrophic cases and 44 per cent of the hypertrophic cases) in that they required more clothing and bedding than other members of their families. Some had always suffered in cold weather; others only since the onset of the arthritis. It is our belief that this symptom is often simply a reaction to illness and does not by itself suggest a glandular disorder.

Those who complained of cold hands and feet were also equally distributed as to type, 48 per cent of the atrophic patients and 50 per cent of the hypertrophic patients. However, this was accompanied in most of the atrophic patients by vasomotor changes such as mottling and swelling, a feature rarely found in the other group. Loss of the normal amount of moisture on the skin, in fact, was found in the majority of the hypertrophic patients. This dryness had to be distinguished from that found with senile atrophy and from the changes due to exposure to cold weather. It involved the hands particularly, but the arms, legs, and feet by no means escaped. The skin was frequently parchment-like, relatively inelastic, and often firmly attached to the flesh beneath. Some patients with atrophic changes of long duration had such skin, but the active cases of this type had characteristically a moist skin.

Nearly all of the patients complained of ease of fatigue. A sense of exhaustion would follow quickly upon even ordinary effort. They would be very tired on rising in the morning, and gather themselves together only slowly as the day wore on. Persistent effort was necessary to keep up with the demands upon them. In the atrophic group this was commensurate with the degree of arthritis and dated from its onset. In the hypertrophic group the striking fact was that these people appeared to be for the most part well nourished and of good physical capacity. Some had carried successfully more than their share of life's burdens. To feel unable to work at the usual speed or for the usual length of time was quite a new experience to them and this was out of all proportion to the extent of the joint involvement; others had been easily tired for years before their arthritis began, but had driven themselves to carry on with an average job. Symptom 5 in Table I illustrates this further. In general, it appeared that the patient with atrophic arthritis felt fairly well apart from his joint pain, unless he was wasted from repeated exacerbations over a long period of time. Certain patients with hypertrophic arthritis, however, had less disability from joint pain, and, though appearing well, would complain of being below par. Others in this group, besides being below par and complaining of ease of fatigue had headaches, and pains referred to muscles and bones as well as joints, with very few objective signs of hypertrophic arthritis. We have considered them under the heading of "Arthralgia."

Paresthesias of the extremities were reported by half of the hypertrophic patients and one-fourth of the atrophic patients, being noted as numbness,

tingling, burning, or prickling. They were sometimes more pronounced in the involved fingers, but not limited to them, or the hand. Factors which tended to decrease circulation, such as exposure to cold, tended to produce them. They were, therefore, most common after exposure to cold and on waking from sleep, and tended to improve, along with characteristic morning stiffness, as the circulation became more vigorous. They were less commonly met with in the patients with very active atrophic arthritis, occurring more frequently in those of that type with long standing changes.

In one-fourth of the atrophic patients the finger nails were described as ridged, broken and brittle; and in a few there was a tendency for the hair to be excessively dry and to fall out. These symptoms, while twice as common in the hypertrophic individuals, were reported in less than half. The greater average age of this group would account for some of the difference, but in certain cases the findings were so striking and the onset so closely linked with the rheumatic process that we were inclined to feel that both could be attributed to hypothyroidism. It is our impression that the incidence of these signs will increase with closer observation.

Edema about the eyes exhibited the same group variation, (24 per cent of the atrophic cases and 38.6 per cent of the hypertrophic cases). Such eyes were those with an appearance of puffiness or pouchiness and congestion about the lids and narrowing of the palpebral fissure. No factor of cardiac or renal disease was present to account for it.

Subcutaneous edema, elsewhere than about the eyes, was noted very infrequently and about equally in the two groups. It was observed in the skin of the ankles, hands (dorsum), and face especially. It was only occasionally due to heart disease with congestive failure. Localized waxy areas of myxedema and fat pads over the shoulders, as mentioned by some writers in the literature quoted above, were rarely seen.

Thirty-five per cent of the patients with atrophic arthritis were definitely overweight, that is, more than 15 per cent over their ideal weight, while 66 per cent of the hypertrophic patients were obese. This is further evidence suggestive of a different constitutional background of health and interaction with disease. The typical rheumatoid patient is wasted and eats listlessly of a restricted diet. The hypertrophic person is usually robust or buxom and blessed with a generous appetite. As pointed out elsewhere (Monroe and Hall[18]) starch indigestion is more common to him also.

Enlargement of the thyroid gland was reported as having been present in 4 cases in each group at some time. One in each group had been subjected to subtotal thyroidectomy for hyperthyroidism in the past; in one hypertrophic arthritis followed directly. Three had adenomas and three had colloid goiters. None had increased metabolic rates (over plus 10) at the time of this investigation.

Thirty-six per cent of the women with atrophic arthritis dated the onset of their disease at the menopause or a year before or after it. This was true of the majority of those women in the hypertrophic group, where 64 (57 per cent) so reported.

Basal Metabolic Rates.—There were 206 determinations of the basal meta-

bolic rate made on 106 patients with atrophic arthritis, and 216 were made on 108 patients with hypertrophic arthritis. No tests were done on 44 of the first group, 6 of which were private patients; and none on 42 of the second group, 5 of which were private patients. The test was almost routine in office practice because of the author's interest in this problem. In some of the clinic cases it was omitted because of the difficulties encountered in such an ambulatory group. In the hospital wards it was done chiefly when there was reason to suspect an abnormal result. No case was excluded for lack of this test. The ward and clinic cases were done in the hospital metabolism laboratory by a technician and an assistant resident in medicine. The Benedict-Roth apparatus was used. The private cases were done by a trained worker using the same apparatus. It was unusually difficult to secure satisfactory curves and checks on tests done on these patients. This was due not to temperature or pulse elevation but to inability to secure good relaxation. Many people came from a distance and had to be allowed to rest for an hour or more before they could breathe quietly. Pain, discomfort, and nervous tension were sometimes great enough to invalidate the test or cause it to be given up. Repeated tests usually showed lower rates, when these factors were overcome. The errors are all on the side of increased oxygen consumption. Better results might be obtained under improved conditions and should show fewer readings over plus 10 per cent. Table II shows the results, with percentages on each bracket.

TABLE II

THE LOWEST BASAL METABOLIC RATES IN 106 CASES OF ATROPHIC ARTHRITIS (206 TESTS) AND 108 CASES OF HYPERTROPHIC ARTHRITIS (216 TESTS)

B. M. R.	ATROPHIC ARTHRITIS		HYPERTROPHIC ARTHRITIS	
	NO. CASES	%	NO. CASES	%
+26 to +29	2	1.8	0	0
+21 to +25	2	1.8	1	0.9
+16 to +20	6	5.6	1	0.9
+11 to +15	2	1.8	5	4.5
+ 1 to +10	18	17.0	16	14.8
0 to −10	38	36	31	28.7
−11 to −15	19	17.9	17	15.7
−16 to −20	15	14.1	20	18.5
−21 to −25	3	2.7	10	9.3
−26 to −29	1	0.9	7	6.4
Totals	106	99.6	108	99.7

It is at once apparent that the majority of cases fell within normal limits. Between rates of plus 10 and minus 10 there are 53 per cent of the atrophic group and 43.5 per cent of the hypertrophic group; between plus and minus 15 there are, respectively, 72.7 per cent and 63.7 per cent. In the abnormal ranges, the differences are rather striking. Of the atrophic cases, 9.2 per cent had rates above plus 16 as compared with 1.8 per cent of the hypertrophic cases. Below minus 16 are found 17.7 per cent of the atrophic cases, and 34.2 per cent of the hypertrophic cases; while below minus 20 there are only 3.6 per cent of the atrophic cases and 15.7 per cent of the hypertrophic cases. There is, thus, a

definite tendency for the hypertrophic patient to have a metabolic rate below normal. Hypometabolism is found in the other type, but to a much smaller extent. This is brought out in Chart 1.

Results of Thyroid Gland Administration.—No simple experiment, in which no other treatment than thyroid gland substance was given, was possible to carry out in our series of cases, except to a certain extent in a few instances. While presentation of such a group of cases affords clear proof of the utility of this medication, as will be shown in a subsequent paper, they are few in number and are not entirely similar to the average case of arthritis with which one is confronted. We were forced to treat the patient as a whole. In general, this consisted in prolonged bed rest at home or in a hospital; a diet rich in vitamins, usually with cod liver oil, viosterol, yeast, orange or tomato juice added; attempts to correct postural and mechanical defects; the search for and care of infections; and attention to concomitant diseases, as necessary. Obviously, the question as to which element in such a manifold program was responsible for any subsequent improvement in the individual patient was often

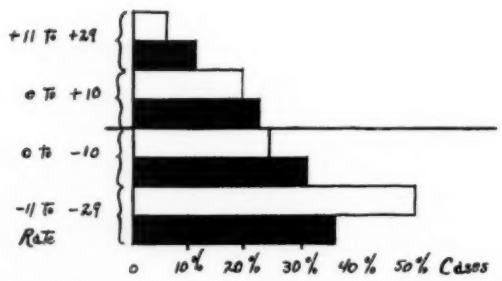

Chart 1.—The lowest basal metabolic rate determination in 107 cases (206 tests) of atrophic arthritis (shaded areas), and 108 cases (216 tests) of hypertrophic arthritis (unshaded areas). While about half of the cases lie between ±10, there is a definite tendency for the rate to be low in the rest, particularly in the hypertrophic type.

extremely difficult to answer. The rôle played by thyroid medication was settled separately for each case by both authors, one of whom, having had little association with many of the patients, acted to a degree as a neutral observer.

Thyroid extract was not given routinely. It was withheld from 5 atrophic arthritic patients and 2 hypertrophic arthritic patients in the private group, and from 42 atrophic and 32 hypertrophic patients in the ward and clinic group. This means that therapeutic tests were almost universal in one group, but not in the other. Harmful effects were limited to sensations of dizziness, nervousness, tension, headache, and insomnia. They promptly disappeared when the thyroid extract was omitted and serious symptoms were not encountered. This may have been due to our policy of giving small initial doses (one-quarter grain desiccated thyroid once or twice daily).

It was decided that thyroid extract had no effect when there was no demonstrable improvement following its use. It was described as of "temporary value" when it seemed to accelerate improvement for a short time, but was without consistent effect throughout the period of observation. Its effect was "good" when it was of unquestioned value in our and the patient's estimation over and above all other elements of treatment for a considerable period of time.

These beneficial effects require some description. Objectively, there was a change from a dry to a moist skin, and an increase in pliability and wrinkling where it had been abnormally firm and thick. Congestion about the eyes and throat improved. Those who were obese lost weight, particularly about the abdomen, hips, and ankles. The affected joints were usually more normal in size due to a loss of boggy swelling; their function was measurably improved. The subjective benefits were often out of proportion to the immediate improvement in physical signs. The patient felt better at once and was better organized nervously, so that he bore his infirmities with great equanimity. Joint sensitivity abated quickly. Physical activity without pain was increased even before there was any demonstrable change in the joints that we could measure. There was less sensitivity to cold and less trouble with cold hands and feet.

Table III shows the results of thyroid therapy in this series of cases. No effect was observed in 71.8 per cent of the atrophic group (46 per cent of the private cases and 87.5 per cent of the hospital cases), and in 31.0 per cent of the

TABLE III

THE EFFECT OF ADMINISTRATION OF THYROID EXTRACT TO 103 PATIENTS WITH ATROPHIC ARTHRITIS AND 116 PATIENTS WITH HYPERTROPHIC ARTHRITIS

RESULT	ATROPHIC ARTHRITIS				HYPERTROPHIC ARTHRITIS			
	PRIVATE CASES		HOSPITAL CASES		PRIVATE CASES		HOSPITAL CASES	
	NO.	%	NO.	%	NO.	%	NO.	%
No effect	18	46.1	56	87.5	13	24.1	23	37
Temporary good effect	8	20.5	4	6.3	5	9.2	18	29
Good effect	13	33.3	4	6.3	36	66.7	21	34
Totals	39		64		54		62	

hypertrophic group (24.1 per cent of the private cases and 37 per cent of the hospital cases). Beneficial effects, both temporary and good, were obtained in 28.2 per cent of the atrophic group and in 69 per cent of the hypertrophic group.

The difference in value of the drug in the hospital cases as compared with the private cases can be explained in several ways. In the latter group the patients were observed for a longer period of time by one group of physicians particularly interested in arthritis, and on the watch for the suggestive signs and symptoms of glandular deficiency pointing to a need for a basal metabolic rate determination. In the former group, the patients were seen for shorter periods of time by a varied group of internists; they had more complicating factors of disease and were less able to follow strict treatment.

It is evident that thyroid was of real value in one-third of the private cases of atrophic arthritis and in only one-sixteenth of the ward and clinic cases. In the hypertrophic cases there were definitely fewer failures, more temporary effects, and many more good effects. Where good effects were obtained, they appeared soon after the drug was given and disappeared soon after it was stopped; it seemed to meet a real deficiency. On the other hand, the temporary good effects seemed more explicable on grounds of stimulation or other action

of the drug, as improvement was accelerated by its use but continued after its withdrawal. In this group, too, care in the application of other factors of treatment had much to do with the way thyroid acted. Temporary value was noted more in the cases which could take adequate rest and diet. In other cases, thyroid seemed to be of no help, and sometimes caused an increase in symptoms. Patients with active or chronic infections were rarely able to take it.

As to the effect of the administration of thyroid on the basal metabolic rates of this group of patients, our data are rather inconclusive. Many patients had only one test performed because they were not studied intensively from this standpoint (as in the private hospital cases) or because of the difficulty in getting outside patients back for the test, and the limited facilities of the metabolism laboratory. In those who had more than one test there was a tendency to omit continued observation when improvement set in, for the same reasons. Demonstration of the fact that arthritic patients with definitely low metabolic rates responded to thyroid therapy, with a uniform return of the rates to normal, would be valuable evidence that hypothyroidism is present, for it is known that the metabolic rate is quite sensitive to thyroid when it is needed, but that large doses will not affect it when it is not needed.

Of 56 cases of hypertrophic arthritis that had two or more metabolic rate determinations, 25 (44 per cent) showed a rise of the rate to normal, from an average initial rate of minus 13 to an average final rate of plus 0.9. Twenty-five (44 per cent) showed no definite change from the first on subsequent examinations, although the figures are rather unsatisfactory; and 6 (11 per cent) showed later a lower rate than at first. The fallacy of excluding hypothyroidism on the basis of one normal rate determination is shown by the fact that 13 whose first test showed readings from plus 6 to minus 11 later showed lower rates (minus 17 to minus 28) and still later normal readings (plus 10 to minus 11) following thyroid therapy.

Of 60 cases of atrophic arthritis that had repeated metabolic rate determinations, 16 (26 per cent) showed a rise of the rate to normal, from an average initial rate of minus 14 to an average final rate of plus 4. Twenty-nine (48 per cent) showed no definite change, and 15 (25 per cent) showed lower final rates than the initial ones. Thus, the figures in this group are less striking than in the hypertrophic group, and in agreement with other differences before noted.

Data from a few closely followed cases with the stigmata of hypothyroidism and low initial metabolic rates, which show a loss of symptoms and normal rates after thyroid therapy, have convinced us that if it had been possible to have more metabolic studies in comparable cases, the results would have been similar in a high percentage. The cases which showed no change in metabolism after thyroid therapy were for the most part those which lacked more of the stigmata of hypothyroidism. Some of the latter improved on thyroid therapy, probably due to the nonspecific effects of the drug, while others did not respond at all.

Further evidence of the value of thyroid therapy is presented in Charts 2 to 5, in which the general results of treatment have been plotted. This assessment was arrived at by grading each case on a basis of one to four, from two standpoints, the extent of joint involvement and the amount of pain and disability, after the practice of the Mayo Clinic. The number of diseased joints was estimated by physical

examination, since x-ray films of the whole skeleton were not feasible. A person with one knee or a few fingers alone involved was rated as one; with both hands and fingers, wrists and a few other joints (perhaps one-half of the total joints of the body), as two; and with apparently all joints, as four. Pain of a mild sort, not

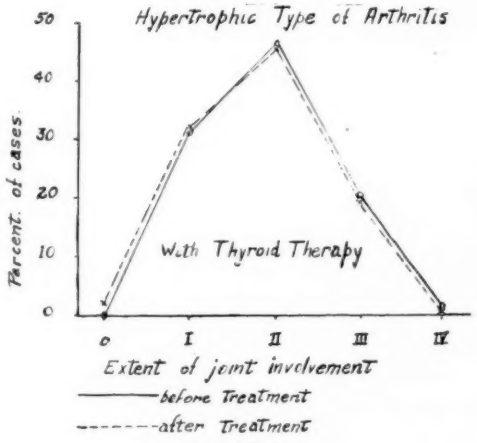

Chart 2-A.

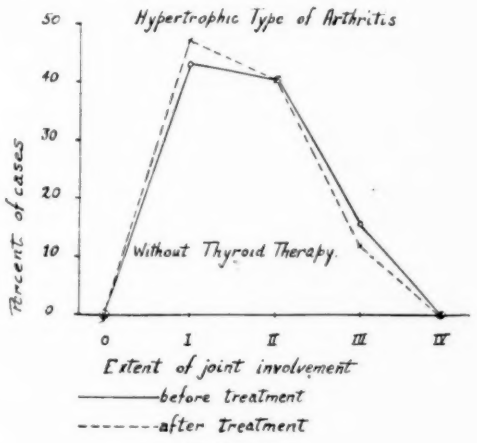

Chart 2-B.

Chart 2-A.—The extent of joint involvement in 118 cases of hypertrophic arthritis before and after treatment which included thyroid substance. For explanation of degree of extent of involvement see text.

Chart 2-B.—The extent of joint involvement in 32 cases of hypertrophic arthritis before and after treatment which did not include thyroid substance. For explanation of degrees of extent of involvement see text.

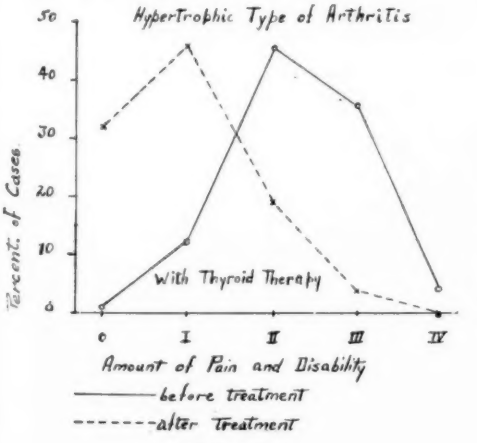

Chart 3-A.

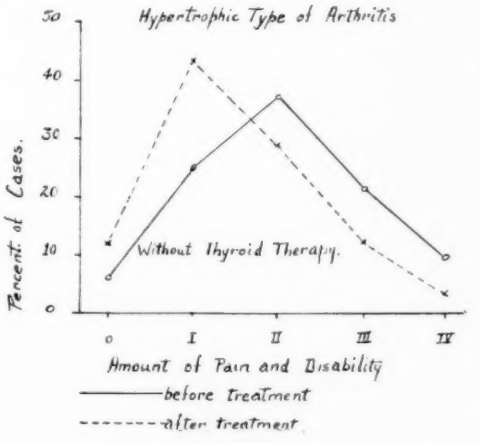

Chart 3-B.

Chart 3-A.—The amount of pain and disability in 118 cases of hypertrophic arthritis before and after treatment in which thyroid was included. For explanation of the rating of pain and disability see text.

Chart 3-B.—The amount of pain and disability in 32 cases of hypertrophic arthritis before and after treatment in which thyroid was not included. For rating of amount of pain and disability see text.

causing limitation of activity, was estimated as one; pain and disability enough to cause moderate restriction, two; complete prostration and helplessness, four. The hospital and clinic cases were followed for an average of one year and three months, the extremes being one month and seven years. The private cases were followed for an average of two years. Patients treated with thyroid were

grouped in one series of charts, to contrast them with those who did not have thyroid.

Charts 2-A and 2-B show that in the hypertrophic cases the extent of involvement did not change as a result of treatment. No joints with definite degenerative,

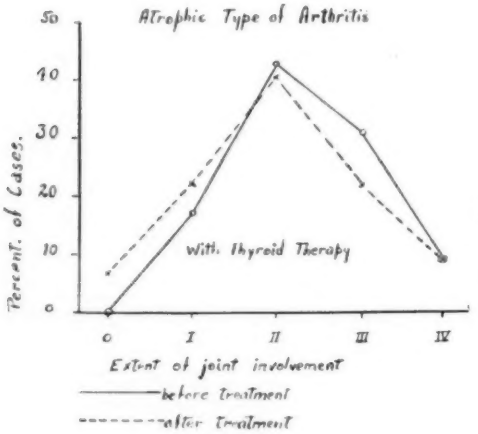

Chart 4-A.

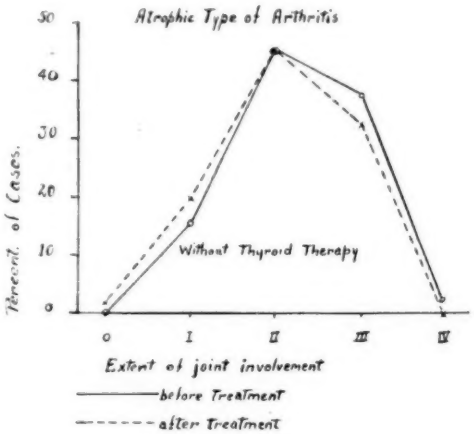

Chart 4-B.

Chart 4-A.—The extent of joint involvement in 106 cases of atrophic arthritis before and after treatment in which thyroid was included. For explanation of degrees of extent of involvement see text.

Chart 4-B.—The extent of joint involvement in 46 cases of atrophic arthritis before and after treatment which did not include thyroid. For explanation of degrees of extent of involvement see text.

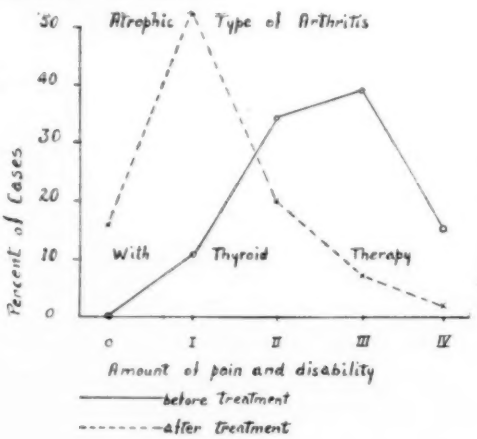

Chart 5-A.

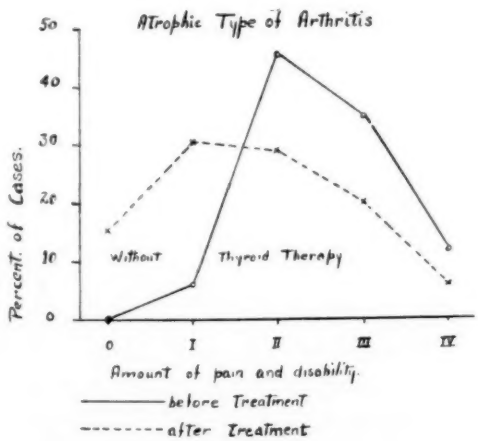

Chart 5-B.

Chart 5-A.—The amount of pain and disability in 104 cases of atrophic arthritis before and after treatment which included thyroid. For rating of amount of pain and disability see text.

Chart 5-B.—The amount of pain and disability in 46 cases of atrophic arthritis before and after treatment which did not include thyroid. For rating of the amount of pain and disability see text.

osteoarthritic lesions were restored to normal, of course. On the other hand, the close approximation of the two lines before and after treatment shows that, with or without thyroid, the disease appeared to be arrested, at least for the duration of this period of observation. The similarity of the two groups of cases with regard to severity is also to be seen at a glance. Those who received thyroid were rated as

being mostly "one" and "two" in extent of involvement, and so were those who did not receive thyroid.

Charts 3-A and 3-B show that pain and disability were considerably decreased after treatment in the hypertrophic group, and without the use of analgesics. Those who did not receive thyroid (Chart 3-B) were rated as mostly "one" to "three," and "zero" to "two" later. However, the separation of the lines before and after treatment is much more striking in the group which took thyroid; they were for the most part "two" and "three" before, a bit more severe than the other group, and became mostly "zero" to "one" afterwards. There were many patients who secured complete release from pain, and while joint motion in some others continued to be limited, functional activity was often extraordinarily increased. Very few patients in this group continued to have extreme disability.

Charts 4-A and 4-B show, in the atrophic arthritic patients, the same tendency for the degree of involvement to be fixed, though there is a suggestion that some restoration toward normal has taken place. There is more separation of the lines and shift to the left here than in Charts 2-A and 2-B. The general onset of the disease process is also apparent as before.

Charts 5-A and 5-B show a more favorable balance for the thyroid treated atrophic cases as against the nonthyroid treated cases than one might expect from Table III. While there is considerable improvement apparent without thyroid, most of the cases remain in grades "one," "two," and "three" of disability and pain. Very few cases are left in grade "three" where thyroid was given, and many more have reached grade "one."

DISCUSSION

This analysis of 300 cases of chronic arthritis suggests that some degree of thyroid gland deficiency may be a common contributing factor, since the symptoms and physical signs usually attributed to such deficiency were found in a large number, since there was a definite tendency for the basal metabolic rate to be low, and since it seemed clear by all tests that thyroid gland substance added to the general treatment was of value in many. The evidence was consistently more impressive for the hypertrophic type of arthritis than for the atrophic type. Hypothyroidism is, therefore, worth searching for and treating in this disease.

Each of the symptoms and signs considered in the literature to be typical of thyroid gland deficiency, and listed by us in our series of patients are found individually, of course, in a variety of diseased states and can be caused by various factors. But the frequent occurrence of several of them together, as shown in Table I, creates an impression that a true lack of thyroid secretion is responsible. They are much more frequent in the hypertrophic type of arthritis than in the atrophic type, and it is in that type that the metabolism rates were most frequently subnormal and that the glandular medication proved to be of more consistent and permanent value. As has been shown, one who develops hypertrophic arthritis is quite a different type of individual from the one who develops atrophic arthritis, and the subsequent improvement in his general condition and in his joints is definite, often striking, and frequently out of all proportion to what one would expect from the correction of such moderate deficiencies.

We have noted frequently, although we have no figures to offer, an appearance of congestion and edema about the tonsils and pharynx and the uvula in these patients, particularly those with hypertrophic arthritis and evidence of hypothyroidism. The eyelids often share in this process. It seems quite probable that it represents an infiltration due to thyroid gland deficiency, as noted by Foster.[9] The redness of the anterior pillars and enlargement of the tonsils has often led to their removal as a focus of infection, usually without benefit to the patient. We have seen the same congestion of the throat in patients who have had adequate tonsillectomy.

The difficulties in securing valid basal metabolic rate determinations in our patients, particularly in the large ambulatory group, has been mentioned. The arthritic individual is typically one who cannot understand that he has a general disease, but feels that he is well except for his arthralgias. Consequently, he does not give up to it, and drives himself to keep going from fear of disability and the accusation of being a neurotic. This nervous tension is reflected in the irregularity of the kymograph tracings when he submits to a metabolism test. Pain also prevents proper relaxation, as well as activity before the test. If the patient comes to the laboratory from any distance, he requires more time to rest than other types of patients. A normal metabolism report in an arthritic patient, therefore, particularly if it be the first test, cannot be taken as a true reading or as excluding hypometabolism. The error is always in the plus direction, not in the minus one. In many patients with a moderate degree of arthritis there is a marked fall in the reported metabolic rate, often 20 per cent, after a few days' rest and release from pain. Swaim has emphasized this also. We feel sure that our tabulated results of the metabolism determinations in this series of patients err in being in general too high. If all the patients could have been tested only after a period of hospitalization, perhaps more would have been found to be subnormal, as was the case in our hospitalized private patients.

Critical examination of metabolism studies has led many writers to distrust the metabolism rate as an infallible index of the activity of the thyroid gland. Benedict[20] has shown that body weight and the state of nutrition influence the rate importantly. A depleted, underweight individual may have a minus 20 reading, and an obese person a plus 20 reading. Exhaustion may cause a low reading, and recovery from acute infections may be attended by low readings, as shown by Gordon and Rabinowitch.[21] Several have noted that a person whose habitual level is minus 10 may have hyperthyroidism with a rate of only plus 15 or plus 20. Similarly, it is probable that one whose usual level is plus 10 may have hypothyroidism or myxedema with readings of only minus 10 or minus 15. It seems fair to say that the metabolic rate is nonspecific and only one sign of thyroid activity, to be considered with the clinical signs and symptoms pointing to hyperactivity and hypoactivity. Recognition of this fact may serve to place hypothyroidism, as distinct from myxedema, on a more clearly established basis than it has enjoyed in the past.

The mode of action of thyroid gland extract in relieving the general and local condition in patients with chronic arthritis may also be both specific and nonspecific. The benefit in any particular case from the use of thyroid therapy is, of course, no proof that hypothyroidism or myxedema are present. Adrenalin has a

wide range of usefulness apart from adrenal insufficiency. Similarly, thyroid extract aids in reducing weight, increases the circulation and is concerned with calcium metabolism. We are convinced that it meets and controls a real deficiency in certain cases of arthritis, as our figures show. We cannot prove that it may not act in some of these cases as a nonspecific agent, however.

If myxedematous infiltration of the joint tissues occurs, it should act to clear up such collections as it does those in other body tissues, such as the heart, kidney, peritoneal cavity, eye, and skin. The senior author has had several cases of mild arthritis with joint swellings which disappeared after thyroid extract was given, and other patients with arthralgias and symptoms of deficiency, but no joint change, who were relieved in a similar fashion.

This is the most direct evidence which we have had that the drug can act locally on the diseased joints, and substantiates the clinical observations which we have quoted above from the literature. We have been unable to find any mention of pathologic examination of the joint structures in myxedematous patients. Autopsies are rarely done in this condition and the joints are usually spared. That they should escape so generalized a process seems improbable. The bony changes in cretinism, however, have been well described and seem to be due to poor circulation and hence to poor local nutrition.

Poor nutrition of the joints has long been considered to be a factor in hypertrophic arthritis. Nichols and Richardson named this type "degenerative," while others use the term "senile." Definite local causes of poor circulation have been described in the form of arteriosclerosis and endarteritis. They apply also to myxedema[9, 10] in which arteriosclerosis occurs prematurely and there is slowing of the rate of blood flow out of proportion to the metabolic rate, retardation of all tissue growth, and often a secondary anemia. It is quite possible that a moderate decrease of nutrition to the joints from any cause may be of more importance than a similar condition in other tissues, due to the trauma to which joints are subjected. Those which are particularly exposed to mechanical stress and strain, the terminal joints of the fingers, the spine, hips, and knees, are the joints of predilection in hypertrophic arthritis. Long continued mild thyroid deficiency may be comparable to long standing dietary deficiencies in causing generalized tissue change. Therefore, it may be that thyroid therapy can act even in the absence of myxedematous joint infiltrations in mild cases of thyroid deficiency and in cases without deficiency by improving the circulation and nutrition.

Thyroid extract also is concerned with the regulation of calcium and phosphorous metabolism. The myxedematous patient has diminished rates of exchange of these substances. This may amount to as much as 40 per cent retention of calcium, as shown by Aub and Bauer.[21] Roentgenograms of his hands show dense bone, while roentgenograms of the hands of hyperthyroid patients show the bone thinner than normal. That the hypertrophic patient is one who shows abnormal deposition of calcium in bones, joints, ligaments, and blood vessels seems more than coincidence, and more than can be explained on the basis of age. Moreover, Pemberton[22] has shown by experiments on dogs, that reduction of the blood supply to the patella leads to abnormal calcification. Thyroid therapy might be expected to bring about a normal exchange of calcium and phosphorus in these arthritic patients.

Care must be exercised in the selection of patients who are to take thyroid therapy. Routine use of it is irrational and must lead to disappointment and even harm, particularly where there is an element of cardiopathy. It is rarely indicated in active cases of chronic atrophic arthritis of short duration. In this group the etiologic factors are more clearly infection, depletion, exhaustion, and dietary deficiencies; the outstanding stigmas of thyroid deficiency are usually lacking; and low metabolic rates here are due to these causes and not thyroid deficiency. In such patients, and in excessive doses in any patient, it may act as a toxic substance and increase joint pain, and should be used with greater caution. Its greatest usefulness is in those arthritic patients who have signs and symptoms of thyroid deficiency; these include the majority of the hypertrophic type and a small number of the atrophic type of long duration and "mixed" or hypertrophic elements.

We have found it wise to accustom ourselves to the use of one brand of thyroid gland preparation only, since the products on the market vary widely in potency. Reid Hunt[23] has shown that this may amount to as much as 2500 per cent. It should also be noted that "fresh" gland substance may be only one-fourth or one-half as potent as the desiccated substance. Textbooks advocate as a small dose one grain of the desiccated gland substance three times a day. This has proved quite excessive in our experience. To begin with, such amounts often lead to toxic manifestations and to fear of the drug on the part of the patient. We, therefore, begin with one-fourth or one-half grain of the desiccated substance daily, and increase it gradually, as the condition of the patient or the metabolic rate suggest.

Toxic manifestations are to be sought for from the start. The first to appear are dizziness and a sense of tension. Then headache is complained of, or a feeling of pressure over the eyes, sleeplessness, emotional instability, malaise or nervousness. Palpitation and tremor have rarely occurred. All these symptoms rapidly disappear when medication is withdrawn. It is important to bear in mind that after a patient has been taking a maximum dose for some weeks, the optimal maintenance dose may become one-quarter of this, or even none at all. It is the general belief that a myxedematous patient needs to take thyroid continuously for the rest of his life. Sturgis[25] has had cases which suggest that this may not always be so. However, where need for the drug is temporary, one should question whether one is dealing with a true thyroid deficiency or with a case which responded temporarily to the by effects of the drug. A simple way to avoid overdosage is for the patient to omit thyroid for the first week of every month. This has the further advantage of acting as an indication for dosage. In this way we have had only transient ill effects, and few of them.

The evidence of the effect of thyroid medication is to be found in the response of the metabolic rate and the condition of the patient. As Swaim[29] and as Peters and Van Slyke[24] have noted, the former is not to be followed blindly. Complete clinical recovery may take place in myxedema on small doses of thyroid without a significant rise in the metabolism, and serious discomfort may ensue if the dose is increased in order to make the rate normal. The same is true in arthritis. There is, indeed, often an initial fall of the metabolic rate to a lower level and then a rise toward normal; this occurred in a certain number of our cases which were closely studied. Swaim has observed this tendency for the rate to fall to lower levels under

treatment, but he observed a return to normal later on thyroid alone in only 20 per cent. A return to normal occurred in 44 per cent of our hypertrophic cases and in 26 per cent of our atrophic cases that had two or more metabolic rate determinations, and over a period of time which suggested that the rise was due to the use of thyroid rather than to a response to general treatment.

Clinically, there is first an increased sense of well being. The patient feels the cold less, often eats with better appetite and sleeps more restfully; there is a relief of tension and he makes more of an effort to carry out instructions. Rarely is there immediate improvement in the joint condition. He improves, as most arthritic patients improve, first in his general health and later in his joints, skin and other tissues. Definite increase in function is usually found, as our charts show. This sometimes is more subjective because of lack of pain and stiffness than objective, for there may be little or no increase in the range of motion. This is in keeping with our idea that the most one can do medically in treating this disease is to arrest its progress. We cannot restore badly damaged joints to normal. Reconstruction by orthopedic measures comes later after arrest. Pain may disappear slowly but the disappearance of pain is sometimes one of the striking effects of thyroid therapy.

Those who would advocate the addition of a particular therapy in the already complicated management of the disease, which is often characterized by spontaneous remissions, must meet the objection that any treatment sufficiently prolonged will always meet this natural stage of arrest. Probably 40 to 50 per cent of arthritic patients recover from their acute attack regardless of treatment. It is, therefore, not easy to be convincing in describing the results of thyroid therapy in them. Our object in treatment should be the control of the disease over a period of years. We have followed enough patients for some years to feel that this gland substance is of especial value in preventing recurrent attacks.

Finally, we wish to repeat what we said in the introduction: "The most successful treatment is that which seeks to determine the entire etiology in each case (of arthritis), to remove or arrest as many factors as may be and to return the joints, and the patient, to the greatest possible degree of normal function." These studies lead us to believe that thyroid deficiency is one, but only one, of these factors. It requires correction along with diet, fatigue, infection, and trauma. We also feel that the nonspecific effects of thyroid are valuable enough to warrant its use in certain other cases of arthritis in which glandular deficiency cannot be proved. This is true particularly for the cases associated with vascular disease, chiefly the hypertrophic arthritis cases and a few of the long standing atrophic cases.

SUMMARY AND CONCLUSIONS

1. Three hundred patients with chronic, nontuberculous arthritis, have been reviewed for the signs and symptoms of thyroid deficiency, the basal metabolic rates, the action of thyroid gland therapy, and the results of medical treatment, which included thyroid substance. Of these, 150 were of the atrophic type of arthritis and 150 were of the hypertrophic type.

2. In the hypertrophic group of patients:

a. The symptoms and signs of hypothyroidism occurred with great frequency, with no other obvious explanation for them.

b. The basal metabolic rates were below minus 10 in 50 per cent, and below minus 15 in 34.2 per cent of 108 patients, even though most of them were determined under inadequate conditions of rest and in the presence of pain. In 42 patients no basal metabolic rate determinations were made.

c. They were for the most part well nourished individuals and no other explanation for the hypometabolism was apparent except for the glandular deficiency.

d. Thyroid gland therapy was of permanent beneficial effect in 49.1 per cent of 116 patients in whom it seemed wise to use it.

3. In the atrophic group of patients:

a. The symptoms and signs of hypothyroidism were met with less frequently and generally would be accounted for on a basis of depletion and undernutrition.

b. The basal metabolic rates were below minus 10 in 35.6 per cent and below minus 15 in 17.7 per cent of 106 patients. No basal metabolic rate determinations were made in 44 patients.

c. Definite glandular deficiency seemed present in only a small number, and there was usually another explanation for the hypometabolism.

d. Thyroid gland therapy was of permanent beneficial effect in 16.5 per cent of 103 patients in whom it seemed wise to use it.

4. Thyroid deficiency appears to be a contributing etiologic factor in certain patients with chronic arthritis, as well as infection, dietary deficiency, trauma, and depletion. Its correction, when present, improves the general well being of the patient, aids in the relief of joint pain and disability, and helps to lay a foundation for permanent control of the arthritis, probably through better joint nutrition.

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CHEMICAL CHANGES IN THE BLOOD OF THE DOG IN EXPERIMENTAL ACUTE PANCREATITIS*

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CLOSE similarity in the chemical changes in the blood of dogs in experimental acute peritonitis and high intestinal obstruction has been noted by Haden and Orr.[1, 2] In experimental peritonitis no constant change was noted in the carbon dioxide combining power, whereas, in high intestinal obstruction there was a constant rise. A similar picture was observed in experimental dehydration, except in the chlorides which showed an increase.[3]

Sweet[4] has expressed the belief that the cause of death in high intestinal obstruction and acute pancreatitis is the same. Others have observed that the cause of death resulting from complete external drainage of the pancreatic secretion is dehydration and acidosis produced by the loss of sodium and chloride ions.[5, 6] Elman and Hartmann[7] found that by total loss of the pancreatic juice through external drainage there resulted an uncompensated acidosis. If vomiting occurred, however, the acidosis was less marked or even replaced by an alkalosis due to superimposed loss of gastric juice as a result of the vomiting.

Since there is some similarity between the chemical changes in the blood in acute high intestinal obstruction, acute peritonitis, dehydration and complete loss of pancreatic juice, we were stimulated to study the chemical changes in the blood produced by experimental acute pancreatitis.

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METHODS

All operations were done under ether anesthesia with aseptic technic. Injections of gall bladder bile, infected gall bladder bile, sodium taurocholate and ether were made either by syringe method, (under minimum pressure) or under 100 millimeters of mercury pressure, directly into the major pancreatic duct (ductus Santorini) without traumatizing the pancreatic peritoneum. The duct was then ligated with silk and severed. Animals were kept in metabolism cages and given water ad libitum.

The following standard blood chemical methods were used. Urea nitrogen was estimated according to the Van Slyke and Cullen modification of the Marshall method;[8] nonprotein nitrogen according to the method of Folin and Wu;[9] blood chlorides on the tungstic acid filtrate by the method of Gettler;[10] the carbon-dioxide combining power by the method of Van Slyke and Cullen;[11] sugar according to the Folin and Wu method,[12] and creatinine by the method of Folin and Wu.[13]

EXPERIMENTAL OBSERVATIONS

Forty-two animals were used in this series of experiments. Six of these animals were discarded because of surgical complications. Detailed chemical studies of fifteen dogs are recorded in Tables I, II, III, IV, V, and VI.

TABLE I

CHEMICAL FINDINGS IN THE BLOOD OF THE DOG IN EXPERIMENTAL ACUTE PANCREATITIS PRODUCED BY INJECTION OF GALL BLADDER BILE INTO DUCTUS SANTORINI

DOG NO.	QUANTITY INJECTED	DAY AFTER OPERATION	BLOOD						REMARKS
			AMOUNT PER 100 C.C.					CO_2 COMBINING POWER	
			UREA NITROGEN	TOTAL NONPROTEIN NITROGEN	CHLORIDES	SUGAR	CREATININE		
			mg.	mg.	mg.	mg.	mg.	vol. per cent	
1	3½ c.c. bile	0	9.3	22.4	460	101	1.5	35.7	Dog died. Pancreatic necrosis
		1	21.0	49.2	470	52	2.2	36.6	
2	5 c.c. bile	0	10.2	25.0	510	87	1.4	36.6	Dog died. Pancreatic necrosis
		1	38.5	62.5	450	55	1.6	40.4	
		2	57.4	88.3	450	52	1.7	44.7	
		3	96.6	143.0	460	124	1.9	44.3	
		4	128.0	167.0	480	133	1.9	44.3	
		6	130.8	273.0	460	112	2.9	47.7	
3	6 c.c. bile under 100 mm. of mercury pressure	0	9.8	28.2	520	98	1.4	38.5	Dog died. Gangrenous pancreatitis
		1	14.0	29.4	510	79	1.2	40.4	
		2	14.9	29.7	530	88	1.2	39.5	
		5	15.8	30.3	500	79	1.2	41.4	
		6	46.7	83.3	510	83	1.4	34.7	
		7	35.0	67.5	510	108	1.7	40.4	
		8	39.2	68.3	490	114	1.5	41.4	
		9	44.1	87.3	500	105	–	40.4	
		10	42.0	83.3	480	108	–	–	

TABLE II

CHEMICAL FINDINGS IN THE BLOOD OF THE DOG IN EXPERIMENTAL ACUTE PANCREATITIS PRODUCED BY THE INJECTION OF MICROORGANISMS INTO DUCTUS SANTORINI

DOG NO.	QUANTITY INJECTED	DAY AFTER OPERATION	BLOOD						REMARKS
			AMOUNT PER 100 C.C.					CO_2 COMBINING POWER	
			UREA NITROGEN	TOTAL NON-PROTEIN NITROGEN	CHLORIDES	SUGAR	CREATININE		
			mg.	mg.	mg.	mg.	mg.	vol. per cent	
4	15 c.c. saline suspension B. coli	0	14.0	28.5	520	86	1.2	39.5	Dog died. Suppurative pancreatitis
		1	49.0	85.3	500	25	1.4	40.4	
5	15 c.c. saline suspension B. coli	0	9.3	23.6	540	82	1.2	39.5	Dog died. Pancreas edematous fat necrosis
		1	21.0	46.8	400	85	1.5	37.6	
		2	53.7	90.3	250	93	3.2	36.6	
6	8 c.c. bile with B. coli	0	12.1	23.8	540	83	1.2	33.8	Dog died. Purulent pancreatitis
		1	28.0	46.0	530	–	1.4	–	
		2	30.3	50.8	500	69	1.5	40.4	
		4	32.2	60.0	540	50	2.4	45.3	
		10	18.6	28.0	500	30	1.8	57.6	
7	19 c.c. saline suspension B. coli	0	30.3	52.6	450	73	2.6	40.4	Dog alive and apparently healthy 42 days postoperative
		1	51.3	107.2	400	83	3.2	41.4	
		2	81.2	135.0	450	44	4.3	39.5	
		5	46.7	107.0	400	48	4.0	41.4	
		11	42.0	72.3	460	52	2.0	35.7	
		16	32.6	48.2	480	50	2.2	37.6	
		25	31.3	107.2	450	62	2.0	34.7	
		30	37.3	78.0	500	72	1.4	37.6	
		42	36.4	58.8	510	62	2.0	39.5	

TABLE III

CHEMICAL FINDINGS IN THE BLOOD OF THE DOG IN EXPERIMENTAL ACUTE PANCREATITIS PRODUCED BY INJECTION OF SODIUM TAUROCHOLATE INTO DUCTUS SANTORINI

DOG NO.	QUANTITY INJECTED	DAY AFTER OPERATION	BLOOD						REMARKS
			AMOUNT PER 100 C.C.					CO_2 COMBINING POWER	
			UREA NITROGEN	TOTAL NON-PROTEIN NITROGEN	CHLORIDES	SUGAR	CREATININE		
			mg.	mg.	mg.	mg.	mg.	vol. per cent	
8	16 c.c. 3 per cent sodium taurocholate	0	12.1	24.8	520	81	1.6	34.7	Dog died in 30 hours. Hemorrhagic pancreatitis, fat necrosis
		1	23.3	56.4	450	20	2.4	43.3	
9	22 c.c. 2 per cent sodium taurocholate	0	11.2	28.0	510	80	1.6	34.7	Dog died. Hemorrhagic pancreatic necrosis
		1	14.0	24.8	500	52	1.2	39.5	

TABLE IV

CHEMICAL FINDINGS IN THE BLOOD OF THE DOG IN EXPERIMENTAL ACUTE PANCREATITIS PRODUCED BY INJECTION OF ETHER INTO DUCTUS SANTORINI

DOG NO.	QUANTITY INJECTED	DAY AFTER OPERATION	BLOOD						REMARKS
			AMOUNT PER 100 C.C.					CO_2 COMBINING POWER	
			UREA NITROGEN	TOTAL NON-PROTEIN NITROGEN	CHLORIDES	SUGAR	CREATININE		
			mg.	mg.	mg.	mg.	mg.	vol. per cent	
10	4 c.c. ether	0	9.3	29.7	530	71	1.3	38.5	Death 10th day. Local abscess
		1	14.0	45.0	460	89	1.2	39.5	
		2	16.3	43.6	480	48	1.4	40.4	
		6	19.6	37.5	550	80	1.5	40.4	
		7	40.1	77.3	500	77	2.0	37.6	
		8	91.0	155.0	480	79	2.1	38.5	
		9	126.0	158.0	500	69	2.0	40.4	
		10	159.7	198.0	520	76	2.6	40.4	

TABLE V

CHEMICAL FINDINGS IN THE BLOOD OF THE DOG IN EXPERIMENTAL ACUTE PANCREATITIS PRODUCED BY INJECTION OF GALL BLADDER BILE INTO DUCTUS SANTORINI

DOG NO.	QUANTITY INJECTED	DAY AFTER OPERATION	BLOOD						REMARKS
			AMOUNT PER 100 C.C.					CO_2 COMBINING POWER	
			UREA NITROGEN	TOTAL NON-PROTEIN NITROGEN	CHLORIDES	SUGAR	CREATININE		
			mg.	mg.	mg.	mg.	mg.	vol. per cent	
11	10 c.c. bile injected at 80 mm. mercury pressure	0	9.3	24.6	510	80	1.2	38.5	Dog killed 30 days postoperative. Pancreas beginning to atrophy
		1	16.3	36.6	510	88	1.4	39.5	
		2	12.1	27.0	520	86	1.4	40.4	
		6	18.6	33.0	520	84	1.4	39.5	
		10	23.3	41.6	490	73	1.4	37.6	
		15	23.3	41.0	510	83	1.2	42.4	
		24	32.6	56.0	480	96	1.4	39.5	
		27	12.1	33.0	500	83	1.4	41.4	
		34	9.3	28.5	520	62	1.4	39.5	
		38	14.0	30.0	530	79	1.2	40.4	
12	8 c.c. bile infected with B. coli	0	9.8	21.8	520	68	1.4	39.5	Dog alive 24 days
		1	24.7	52.8	510	50	2.0	44.3	
		2	20.5	46.8	400	62	2.0	42.4	
		3	14.0	34.5	410	70	1.8	45.3	
		6	10.2	22.4	410	62	1.4	46.2	
		10	9.3	20.8	490	89	1.2	40.4	
		24	9.3	23.0	500	78	1.6	42.4	

The animals receiving gall bladder bile lived longer than those receiving sodium taurocholate. Not all of the animals showed definite blood chemical changes. The dogs receiving sodium taurocholate showed profound hemorrhagic pancreatitis followed by death within twenty-four to seventy-two hours. Neither the amount of pathology in the pancreas nor the blood chemical changes had any definite relationship to the pressure under which the materials were injected. The urea nitrogen and nonprotein nitrogen were increased in 12 of the 21 dogs receiving gall bladder bile, 3 of the 4 receiving ether, 5 of the 8 receiving infected

TABLE VI

CHEMICAL FINDINGS IN THE BLOOD OF THE DOG FOLLOWING INJECTION OF GALL BLADDER BILE INTO DUCTUS SANTORINI. CHANGES WITHIN NORMAL LIMITS

DOG NO.	QUANTITY INJECTED	DAY AFTER OPERATION	BLOOD						REMARKS
			AMOUNT PER 100 C.C.					CO_2 COMBINING POWER	
			UREA NITROGEN	TOTAL NONPROTEIN NITROGEN	CHLORIDES	SUGAR	CREATININE		
			mg.	mg.	mg.	mg.	mg.	vol. per cent	
13	4 c.c. bile	0	13.0	28.2	510	71	1.6	40.4	Dog killed. No gross pancreatic changes
		1	16.3	31.6	450	69	1.6	38.4	
		2	14.0	32.6	480	71	1.4	40.4	
		6	13.5	29.1	530	74	1.6	40.4	
		10	10.2	29.4	520	61	1.3	40.4	
		18	10.2	27.7	550	79	1.2	36.2	
		33	15.8	32.6	510	77	1.2	36.6	
14	3 c.c. bile	0	9.3	28.5	510	83	1.4	38.6	Dog killed. Pancreas appeared normal
		1	11.6	28.5	500	68	1.5	40.0	
		2	11.6	30.0	520	72	1.6	38.6	
		4	9.3	28.5	520	66	1.6	46.0	
		8	8.8	22.7	550	77	1.2	37.6	
		12	14.0	29.7	550	108	1.4	35.7	
		32	9.8	26.3	520	80	1.3	38.5	
15	6 c.c. bile	0	14.9	28.9	550	85	1.4	40.4	Dog died. Pancreatic necrosis
		1	14.0	27.0	540	79	1.2	38.5	
		2	12.1	28.2	530	63	1.2	40.4	
		3	14.9	28.2	540	76	1.3	38.5	
		4	18.2	34.5	540	71	1.4	37.6	

bile, and 2 of the 3 receiving sodium taurocholate. The creatinine was increased in 14 animals. Seventeen animals receiving gall bladder bile showed so change in the chlorides, whereas, 5 of the 8 receiving infected bile showed a definite decrease. Two of the 4 receiving ether and 2 of the 3 receiving sodium taurocholate showed a fall in the chlorides. Nine animals of the entire series showed a rise in the carbon dioxide combining power and the remaining 27 showed no constant change. The changes in the blood sugar were not constant. An increase in the sugar content was noted in 10 animals, a decrease in 14 animals and no change in 12.

SUMMARY

Sterile and infected gall bladder bile, sodium taurocholate and ether, when injected into the duct of Santorini, produced a definite pancreatitis or pancreatic necrosis of varying degree in all cases.

The urea nitrogen and nonprotein nitrogen of the blood showed an increase in approximately two-thirds of the animals.

The chlorides showed practically no change except a slight decrease in 13 instances.

The carbon dioxide combining power showed a slight increase in 9 animals.

Following operation there frequently occurred a decrease in blood sugar followed by hyperglycemia.

The creatinine usually showed either a slight change or a terminal rise, which was not always proportional to the rise in urea and nonprotein nitrogen.

The changes in the nitrogen elements closely simulate those found in experimental peritonitis, acute intestinal obstruction and dehydration. No explanation is offered for the blood sugar changes.

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ACUTE SPONTANEOUS GLOMERULITIS IN THE RABBIT*†

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VARIOUS forms of spontaneous lesions of the rabbit's kidney have been described by many observers who have used this animal for experimental purposes. The one most frequently found is that of focal nephritis, characterized by destruction of tubules, and by an increased amount of connective tissue in which numerous glomeruli may usually be seen in a fair state of preservation. This lesion in its chronic form usually produces marked pitting of the surface of the kidney. Another lesion is one described by LeCount, in which there is a wedge-shaped mass of dilated tubules, with its base at the surface of the kidney. A third type, described by Mallory and Parker, is acute glomerulonephritis; this is characterized by proliferation of the lining endothelium of the capillaries leading to occlusion of them. Several mitotic figures are found in a single glomerulus. The entire tuft is usually enlarged. The lesion is diffuse throughout the kidney. In the epithelium there is formation of hyaline droplets, necrosis, and calcification.

The lesion that I wish to place on record as occurring spontaneously in the rabbit is very much more acute than those mentioned, and occurs only in a small number of glomeruli in any part of the kidney. Not more than four affected glomeruli have been observed in any single section of the kidney.

In a study of the rabbit's kidney covering many years, I have observed these changes in only two animals. Protocols of these two rabbits are recorded herewith.

RABBIT 1.—January 27, 1931, a rabbit weighing 1.4 kg. was catheterized. The urine contained many hyaline casts, a few pus cells and on culture innumerable gram-negative bacilli. Culture of a catheterized specimen of urine obtained the following day disclosed the same conditions. Four days later the animal died, apparently as the result of diarrhea, not as the result of any experimental procedure. At necropsy the urine from the bladder contained many pus cells and many gram-negative bacilli. Cultures were not made from the kidneys. The ureters and pelvis appeared normal except for a few leucocytes in the lining of the pelvis. The kidneys were brownish red, and appeared smooth after removal of the capsule. On gross section there was no evidence of chronic focal nephritis or other change.

Microscopic examination disclosed a number of foci of acute inflammation in the upper portion of the papilla and in the lower portions of the cortex. These portions were not related to the glomerular lesions found scattered through the cortex. Throughout both kidneys the glomerular tufts were filled with large numbers of polymorphonuclear leucocytes. They were uniformly distributed throughout the glomerulus or were present in groups in a few loops. Occasional leucocytes are seen in sections of glomeruli of normal kidneys but not in the number seen in all of the glomeruli of this animal.

The glomerular lesions are described in what appears to be their various stages

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†Work done in the Division of Experimental Surgery and Pathology, The Mayo Foundation.

of development. The most acute lesion is seen in Fig. 1. About 20 polymorphonuclear leucocytes can be counted in one section of the glomerulus, in addition to a considerable amount of nuclear débris of probable leucocytic origin. The structure of the glomerulus has been almost entirely destroyed, only a small rim of tissue in one quarter suggests tuft structure. Most of the glomerular tuft consists of hyaline, pink staining material in which are seen many leucocytes, and large, clear nuclei of the cells lining the tuft. Cells with similar nuclei are seen in large num-

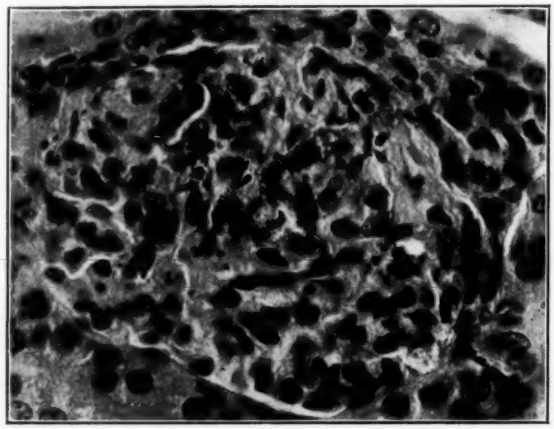

Fig. 1.—Marked infiltration with beginning necrosis.

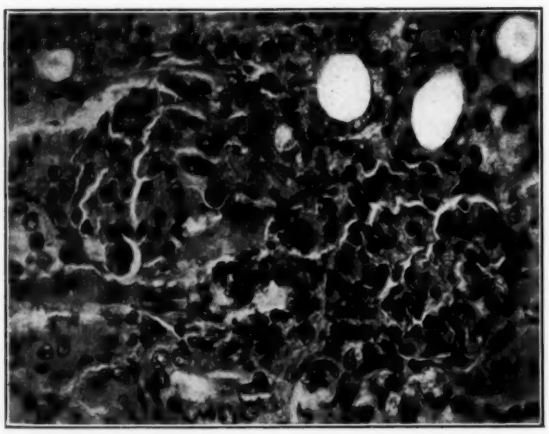

Fig. 2.—Acute polymorphonuclear infiltration with necrosis.

bers in the wall of the thickened capsule on one side. Nuclear débris is most marked in the central portions of the glomerulus. There is no periglomerular infiltration. Fig. 2 represents a second glomerulus, with a large mass of polymorphonuclear cells in one area, and in the opposite end of the glomerulus a few normal capillaries filled with erythrocytes. Two-thirds of the glomerulus has been entirely destroyed. The hyaline material does not contain leucocytes but does contain many large, clear nuclei which increase in number in the capsule. In a closely adjacent glomerulus, there are ten polymorphonuclear cells in the loops that impinge on the glomerular wall opposite the affected glomerulus.

In a little later stage (Fig. 3) the leucocytes have diminished so that only five are seen in a section through an extremely large glomerulus. The destruction of the glomerulus is not as complete as in the first glomerulus described. About one-fourth of the glomerulus appears normal and the loop is filled with erythrocytes; in the other three-fourths the structure has been entirely lost and is made up of hyaline material, nuclear débris, and large, clear nuclei. The capsule is definitely thickened on the surfaces adjacent to the necrosis. Periglomerular in-

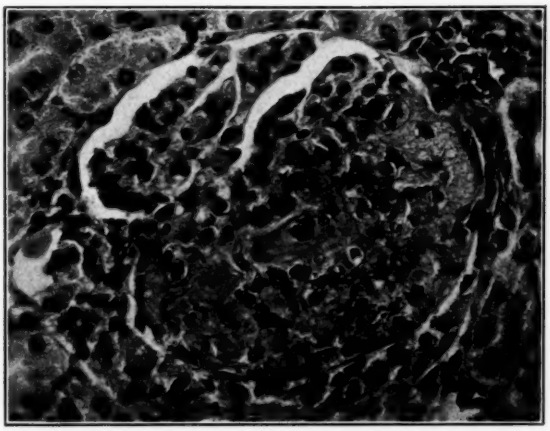

Fig. 3.—Subacute hyaline necrosis.

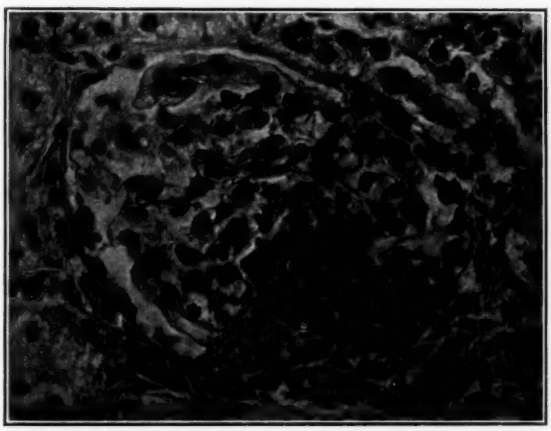

Fig. 4.—Destruction of one-fourth of a glomerulus.

filtration with lymphocytes is present, and is most marked toward a small vein close by.

In still later stages the nuclear débris has almost entirely disappeared, and the glomerulus is made up of a mass of clear nuclei lying in a field of hyaline material completely filling the glomerular capsule. No periglomerular infiltration with lymphocytes was present about the glomerulus. Many sections were carefully searched, but older stages were not found in the kidneys of this animal.

Rabbit 2.—December 2, 1931, a catheterized specimen of urine from a rabbit weighing 1.90 kg. did not contain albumin or casts, and on culture proved

sterile. December 4, 1931, at 3 P.M., the animal received 50 c.c. of 20 per cent sucrose solution containing some dead colon bacilli. The animal was found dead the following morning. The gross examination of all of the organs was negative. I have injected this mixture into many animals, without finding any renal changes similar to those to be described here, and since the interval between injection and death of the animal was too short to have given opportunity for the production of such changes, it seems certain that they were spontaneous in origin.

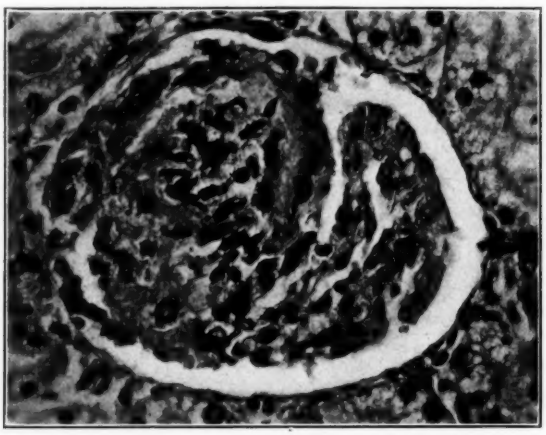

Fig. 5.—Necrosis; no leucocytic infiltration.

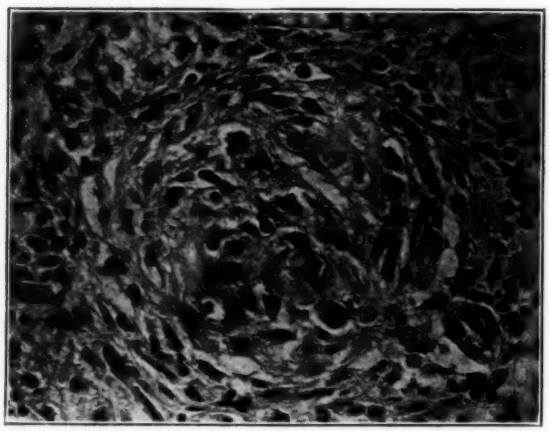

Fig. 6.—Chronic stage.

Sections of the kidney disclosed the typical granular degeneration of the convoluted tubules that is constantly seen after intravenous injection of 20 per cent sucrose. There were a few areas of chronic infiltration in the papilla and cortex. The changes in the glomeruli were definitely less acute than in the first rabbit. They ranged from acute changes in which necrosis of a portion of the glomeruli was seen, to early fibrosis of the glomeruli. In the acute stage, from one-fourth to half of the glomerulus was destroyed, and the normal glomerular structures were replaced by a mass of pink-staining, granular material in which

were numerous small and large granules of nuclear débris. The number of nuclei in the glomerular capsule was definitely increased, and in one area it was made up of four layers of cells. As could be seen in the series of eight sections through this one glomerulus, the lesion was definitely limited to one of its portions. In the first section about half of the glomerulus was replaced by necrosis. In the second and third sections (Fig. 4) the necrosis involved the upper third of the glomerulus, with nuclear fragmentation most marked in the center of the glomerulus. In the fifth and sixth sections the necrosis was limited to about a fourth of the area. In the seventh section there was only a small area of necrosis, and in the eighth the glomerular loops appeared practically normal, but the capsule was thick and infiltrated with a number of lymphocytes. In Fig. 5 another example of acute glomerulitis is shown with destruction of about two-thirds of a glomerulus.

The more chronic stage of the lesions may be seen in Fig. 6. The necrotic material had been almost entirely removed, and in its place there was a concentrically arranged mass of epithelioid cells in which nothing of the former glomerular structure remains. There was some periglomerular infiltration with lymphocytes. This was the oldest lesion found in the kidneys of this rabbit. It is not difficult to conceive of this mass of tissue that has replaced the glomerular tufts gradually shrinking, and leaving the small, completely sclerotic glomeruli that are so often encountered in kidneys of the rabbit which otherwise appear normal.

COMMENT

In many kidneys which appear entirely normal, small, sclerotic glomeruli are found in a parenchyma that shows no evidence of other changes. The number of such glomeruli in any normal-appearing kidney in the rabbit is extremely small. Examples of the terminal stage must be present in larger numbers, and for a longer time, than the acute stages of the process that leads to this sclerosis. It is therefore not surprising that the acute stages of the development of this lesion have not been described heretofore. The change in the glomeruli is so striking that it is not likely to be overlooked, but since it occurs in only one or two glomeruli in the 800 to 1,000 glomeruli of a transverse section of the kidney, it is not likely to be found unless a careful and complete examination of a section is made. It seems probable that the acute and chronic changes observed in the glomeruli of these rabbits might result in the sclerotic glomeruli to be seen in many apparently normal kidneys of the rabbits.

In conclusion I want to emphasize again that these lesions were spontaneous in origin and that in the interpretation of experimentally produced acute glomerular lesions this source of error is to be considered.

DIFFUSE ENDOTHELIOMA OF LYMPH NODES*†

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ACCORDING to Flynn, the term endothelioma was advanced by Golgi in 1869. Chambard, in 1880, wrote a description of primary cancer of the lymph nodes arising from endothelial cells and to him is generally given credit for the discovery of this tumor. He noticed that this neoplastic process may be localized in a node or chain of nodes, or that it might assume a generalized form.

Hoffman and Schottlin, according to Benzinger, confirmed Chambard's finding by a case of their own. Recklinghausen in 1897 recognized primary endothelioma of the lymph node as a distinct tumor.

The next event in the history of this neoplasm was the publication in 1913 of Ewing's monograph on primary endothelioma of lymph nodes. In this paper Ewing reported a series of cases and claimed a definite clinical importance for the disease which had until that time been considered a medical curiosity. He noted that primary endotheliomas of the lymph nodes were commonly confused with such conditions as secondary carcinoma, lymphosarcoma and Hodgkin's disease, and pointed out wherein they differed histologically, anatomically, and clinically.

Oliver, also in 1913, published a paper on the relation of Hodgkin's disease to lymphosarcoma and endothelioma of the lymph nodes. He fully concurred with the opinions expressed by Ewing and included histologic descriptions, without clinical histories, of eight cases. Later authors on the subject of primary endothelioma of the lymph nodes have not added to Ewing's description and have made no new contributions to our knowledge of the subject. At the present time, however, pathologists do not as a rule attach the importance which Ewing did in his monograph to chronic granulomatous processes, notably tuberculosis, as an etiologic factor in the development of this tumor. Since 1913, primary endothelioma of the lymph nodes has been generally considered to be not uncommon. However, the relevant literature of the last two decades is surprisingly scanty considering the clinical importance of this neoplasm. Furthermore, the number of cases reported is remarkably few.

The incidence of the condition varies greatly at different medical centers. In this regard, Piney states that the great divergence of opinion regarding the nature of endothelioma accounts for the fact that some pathologists assert it is quite common, while others make the diagnosis of this tumor seldom and with hesitation. Dijkstra says that there are few tumors concerning which there is such a wide diversity of opinion. He notes that a tumor may be diagnosed as endothelioma by one author; another will diagnose a similar one as carcinoma; while a third will place the same type of neoplasm among the sarcomas or fibromas. He says that this difference in opinion is due in part to the unsolved problem of the structure and origin of endothelial cells.

*From the Clinical Laboratory, Laboratory Center, and Cancer Center, Edward Hines, Jr. Hospital.
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TABLE I

AUTHOR AND DATE OF REPORT	AGE	SEX	SYMPTOMS	DURATION	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	TREATMENT	CLINICAL COURSE	REMARKS
Ciaccio (quoted by Battaglia 1926)	24	Female	Tumor size of hazel nut in right carotid region. Other left side of neck and left axilla.	1 yr.		Syncytio-endothelioma			
Battaglia, F. 1926	60	Male	Frontal headache tumor, painless and movable in left suprahyoid region. Increasing in size. Nodules in pharynx covered with normal mucosa. Wassermann negative.	Several years		Plasmochial endothelioma	Biopsy	Five months or so later patient extremely cachectic. Left cervical, axillary, and epitrochlears of both sides enlarged. Nodules in pharynx so enlarged that patient nourished with milk. Spleen palpable. Inguinal nodes not enlarged. No later report.	At age of thirty left testicle enlarged and painful after a few months. Patient though diagnosed tuberculous had no further disturbance of genitourinary organs and a negative history until onset of present illness.
Guttierrez, A. 1920	33	Female	Numerous subcutaneous nodules infiltrating the external portion of left mammary and left pectoral areas. Mammary glands negative. One nodule below right mammary gland.			1st—subacute diffuse dermatitis. (specific? leprous?). 2nd—"Subcutaneous inflammatory lymphangioendothelioma."	Biopsy. K. I. Neosalvarsan. X-ray. Radium.	In spite of all treatment nodules continue to form (anterior abdominal wall). General condition became bad and patient confined to bed. No further details.	
Flynn, C. 1927	26	Male	Painless enlarging lump at angle of lower jaw.	7 mo.	Adenitis due to infected teeth and tonsils.	Primary endothelioma. Cervical nodes.	X-ray therapy	Not affected by x-ray treatments. Death 2 yr. and 6 mo. after first nodule appeared. No autopsy.	In addition to tumor at angle of jaw, postcervical and supraclavicular nodes became enlarged. X-ray showed enlargement of hilus and bronchial nodes. Metastasis to left breast.

TABLE I (CONTINUED)

AUTHOR AND DATE OF REPORT	AGE	SEX	SYMPTOMS	DURATION	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	TREATMENT	CLINICAL COURSE	REMARKS
McCartney, J. S. 1928	15	Male	*First Admittance* Headache, weakness, pains in legs, dyspnea. Bilateral cervical adenitis (most marked, right side). *Second Admittance* Well marked cervical swelling (left side). Marked weight loss.	4 wk. 1 yr. later	*First Admittance* Syphilis (positive Wassermann). Infected tonsils and adenoids.	*Second Admittance* Endothelioma	*First Admittance* Antisyphilitic therapy. Removal of tonsils and adenoids. *Second Admittance* Biopsy of cervical nodes.	Death a few months later. Partial autopsy.	Partial autopsy revealed large retroperitoneal nodes, and enlarged tracheobronchial nodes, all showing characteristic microscopic picture of primary endothelioma of lymph nodes.
Zaffagnini, A. 1928	73	Female	Painless tumor size of orange in left axilla.	?		Primary endothelioma of axillary glands.	Enucleation of tumor.		Case showed a positive Wassermann. Antiluetic treatment did not affect size of tumor.
Lino, G. 1927	21	Female	Small tumor (size of pea grew to size of hen's egg) in right latero-cervical region. General health excellent.	6 mo.	Neoplasm of lymphatic glands. Endothelioma favored due to slow course.	Primary endothelioma of lymph nodes.	Operative removal of tumor.		

TABLE I (CONTINUED)

AUTHOR AND DATE OF REPORT	AGE	SEX	SYMPTOMS	DURATION	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	TREATMENT	CLINICAL COURSE	REMARKS
Author's Cases									
Case 1 H. W. 1931	32	Male	*First Admittance* Small mass right neck. (Enlarged lymph nodes.) *Second Admittance* Shortness of breath, nervousness. Bilateral enlargement of cervical glands. Thyroid on right side enlarged.	14 mo. 15 mo. later		*First Admittance* Lymphosarcoma *Second Admittance* Slide reviewed with establishment of diagnosis of endothelioma.	Biopsy. Repeated doses of deep x-ray.	No diminution in size of glands following x-ray treatment. Patient home in fair condition.	At time of patient's second admittance he has well marked symptoms of thyrotoxicosis. This latter condition will be treated before surgical treatment of tumor is attempted.
Case 2 W. B. 1931	36	Male	Large mass size of first in left neck with chain of enlarged glands in neighborhood. Small soft mass size of pea on left nasopharynx surrounding auditory tube.	1 yr.	Hodgkin's disease	Biopsy specimen taken from both the gland and mass in pharynx. Diagnosis of endothelioma made.	X-ray and radon implants followed by surgical removal.	No response to x-ray or radon implants.	Patient's general condition exeellent.
Case 3 C. S. 1931	57	Male	Tumor left side of neck.	5 yr.	Sarcoma	*First Admittance* Metastatic carcinoma. *Second Admittance* Restudy of slide establishes endothelioma.	 Deep x-ray.	 No response to x-ray therapy, general condition good.	 In last few years has lost a moderate amount of weight. General condition good. No evidence of metastasis.

TABLE I (CONTINUED)

AUTHOR AND DATE OF REPORT	AGE	SEX	SYMPTOMS	DURATION	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	TREATMENT	CLINICAL COURSE	REMARKS
Case 4 J. G. 1931	62	Male	Complains of a "cold." Shortness of breath, pain in chest, swelling in left supra- and infraclavicular fossae.	?	Malignant new growth of neck and mediastinum.	Biopsy of axillary gland reported as chronic adenitis. Autopsy specimen diagnosed as endothelioma.	Deep x-ray.	No response to x-ray therapy. Symptoms progressive. Death 3 mo. after admission.	Autopsy showed a tumor of anterior cervical nodes and those of the supraclavicular fossa on the left side. The mass weighed 500 grams. A portion of the tumor extended behind the clavicle, expanding in the mediastinum to surround the aortic arch. The left lung was compressed and showed a nodule 1.5 cm. in diameter with the same appearance as the tumor.
Case 5 J. H. 1931	53	Male	Enlargement of neck on left side.	3½ yr.	Hodgkin's disease	*First Admittance 1928* Hodgkin's Dis. *Second Admittance 1931* Endothelioma	X-ray in 1928 with no response. Surgical removal of mass in 1931, save a small substernal nodule which was inaccessible.	No response to x-ray treatment. Since surgical operation, patient is in excellent condition and has gained 8 pounds at the time of this report, 8½ mo. postoperative.	

As noted by Flynn, the condition in its incipiency is a process usually localized in glands readily accessible to surgery. At this stage, surgical intervention offers the best prospect of complete cure.

We present the following 5 cases which we have seen in a period of less than a year at the Edward Hines, Jr., Hospital. It will be noted that the majority of these patients had been under medical treatment for varying lengths of time before the true diagnosis was reached. Table I outlines from a clinical point of

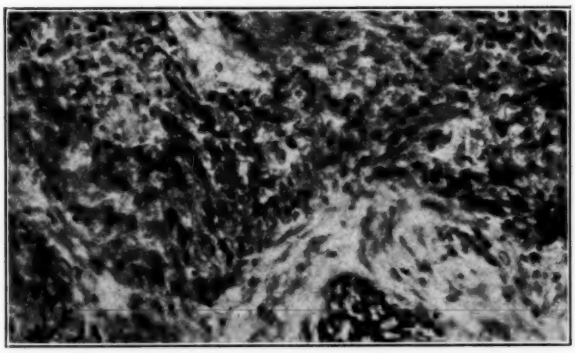

Fig. 1.—Sixteen mm. obj. c.c. 15 × camera 50 cm. × 300. Reduced. Case 1. H. W. Diffuse type. Oval and polyhedral type cells growing in broad sheets, completely replacing lymph node structures. Note the diffuse scarring.

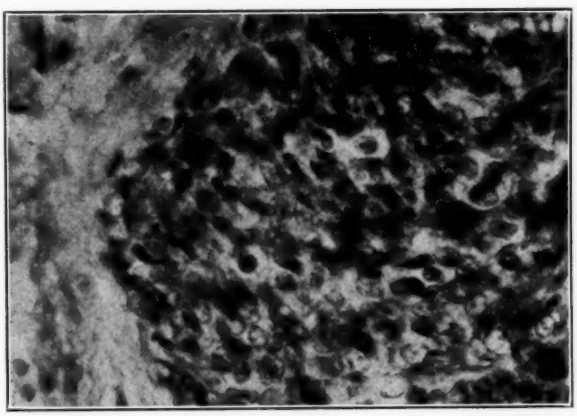

Fig. 2.—Eight mm. obj. c.c. 15 × camera 5 cm. × 600. Reduced. Case 1. H. W. Detail showing indefinite cell outline.

view the cases of primary endothelioma of the lymph nodes which we have been able to collect from the literature since the publication of Ewing's monograph.

Histopathology.—According to Ewing, endothelioma of lymph nodes may be divided into the three following groups:

1. Diffuse
2. Plexiform or perivascular
3. Alveolar

1. Those tumors which show round or polyhedral cells appearing in broad coherent sheets or syncytial masses comprise the diffuse group.

2. The plexiform type shows cells surrounding large irregular centers of cell

detritus or leucocytes. Occasionally cavities appear empty and are lined with flat cells resembling endothelium. Ewing interprets these cavities as derivatives of the original sinuses of the node but is not convinced that this is their sole origin. When the sinuses are highly developed, the tumor cells cling to the arterioles of the septa producing a characteristic perivascular arrangement which recalls the structure of a perithelioma. In the plexiform and perivascular tumors the cells usually assume a spindle form but readily change to polygonal or pavement types.

3. The alveolar type presents many difficulties of interpretation. It is seldom possible to trace the tumor cells to any structure of the original lymph node, and

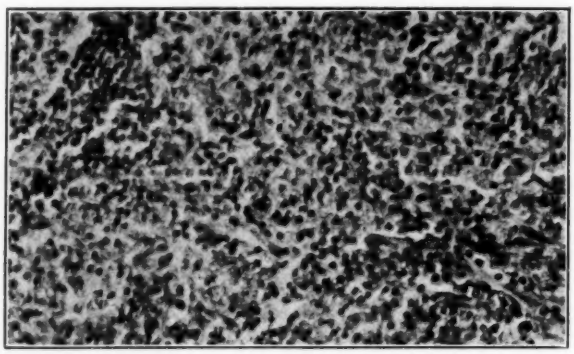

Fig. 3.—Sixteen mm. obj. c.c. 15 × camera 50 cm. × 300. Reduced. Case 4. J. G. Replacement of lymphoid structure by diffuse growth of tumor cells. Case previously diagnosed as chronic adenitis.

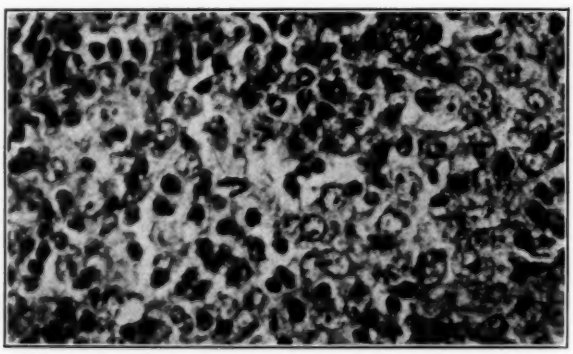

Fig. 4.—Eight mm. obj. 15 × camera 50 cm. × 60. Reduced. Detail showing vesicular character of cells arranged in broad sheets.

the cell groups often resemble those of secondary carcinoma. Thus the diagnosis of this type of endothelioma requires full clinical and anatomical data and must often remain uncertain. It is this type of tumor which has hitherto appeared in the literature and received considerable recognition as true endothelioma of lymph nodes. One of Ewing's cases demonstrates furthermore that endothelial proliferation associated with granuloma may early assume a form closely resembling alveolar endothelioma.

Clinical Diagnosis.—Primary endothelioma of the lymph nodes may suggest from the clinical appearance acute infectious adenitis, tuberculosis, syphilis, lymphosarcoma, Hodgkin's disease, and, above all, metastatic carcinoma. Acute

infectious adenitis will usually show infected areas draining into the affected nodes. Pain and fluctuation eventually are characteristics of the condition. In this connection we may remark that tonsils and adenoids have been regarded as the source of adenopathy in several recorded cases of endothelioma of the cervical nodes, notably those of McCartney and Flynn.

Tuberculosis of glands usually occurs in patients whose history reveals an antecedent tuberculous infection and is characterized by a tendency to caseation and sinus formation. The characteristic temperature elevations and other sys-

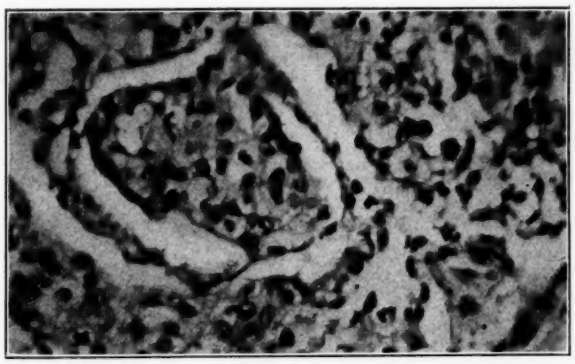

Fig. 5.—Sixteen mm. obj. c.c. 15 × camera 50 cm. × 300. Reduced. Case 2. W. B. Note the alveolar like arrangement of cells.

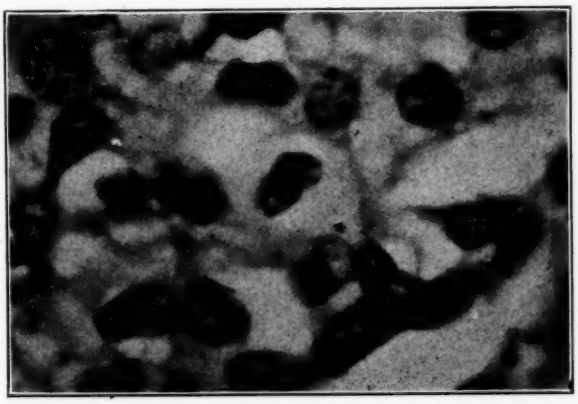

Fig. 6.—Four mm. obj. c.c. 15 × camera 50 cm. × 1200. Reduced. Case 2. W. B. Detail of alveolus revealing a cloudy indefinite cell cytoplasm and the highly vesicular character of the nucleus.

temic symptoms of tuberculosis may be expected. Flynn writes, in this regard, that when tuberculosis is manifested in cervical lymph nodes, it appears first in the submaxillary and soon afterward in the cervical nodes in both sides of the neck, growing slowly, and that gradually the postcervical, supraclavicular, scapular, and bronchial nodes become affected. On the other hand, in primary endothelioma of lymph nodes, the primary tumor is usually localized in one chain, usually the anterior cervical, on one side.

Syphilis is usually suggested by the history and Wassermann reaction. The Wassermann, however, may lead one astray as noted in the cases reported by

McCartney and Zaffagnini, in which a positive Wassermann reaction and an endothelial tumor of the lymph nodes were coexistent.

Lymphosarcoma is characterized by its rapid course and early involvement of associated chains of nodes. This tumor responds to radiation therapy.

Primary endothelioma of lymph nodes is frequently diagnosed clinically as metastatic carcinoma. This is easily understood when we note, as shown by Crowe and Baylor, that in a large series of cases, neck tumor is the first sign of carcinoma, primary in the nasopharynx, mouth, or accessory sinuses. This fact is also stressed by Quick and Cutler. As stated by these latter authors, the failure

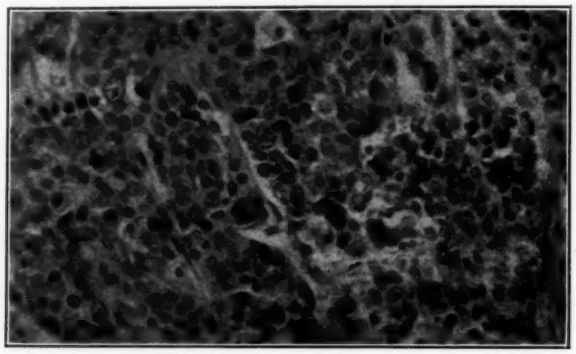

Fig. 7.—Sixteen mm. obj. c.c. 15 × camera 50 cm. × 300. Reduced. Case 3. C. S. Cells growing in columns with an arrangement resembling secondary carcinoma.

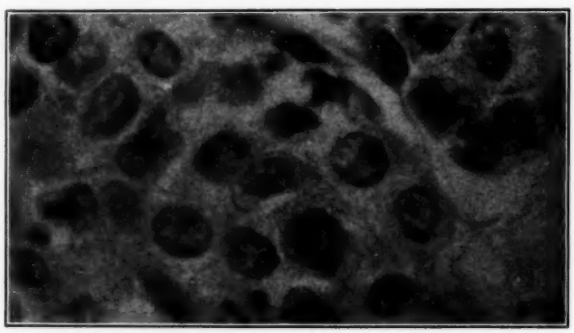

Fig. 8.—Four mm. obj. c.c. 15 × camera 50 cm. × 1200. Reduced. Case 3. C. S. Detail of the invading cells. Note the mitotic figure.

after careful search to demonstrate a primary neoplasm, the rapid course of carcinoma, and the fact that it is radiosensitive should serve to distinguish it from endotheliomas.

Primary endothelioma of lymph nodes differs clinically from Hodgkin's disease in that in the latter a generalized adenopathy usually ensues shortly after the appearance of the cervical tumor. Hodgkin's disease is furthermore characterized by remissions while the course of endothelioma is slowly and steadily progressive. Hodgkin's disease readily responds to irradiation.

We would conclude from a study of our own cases and from those reported in the literature that there are important distinctive features which are characteristic of primary endothelioma of lymph nodes. While, as noted by Quick and Cutler,

endothelioma of lymph nodes may occur as a systemic involvement of many nodes or as single or multiple tumors of the cervical, axillary, or other lymphatic chains. Typically the primary tumor involves and is localized in the anterior cervical group of nodes. The individual glands are usually described as remaining more or less discrete and are found to be rather elastic on palpation. The tumor mass is rarely painful.

The long duration of the clinical course with absence of systemic involvement, above all the lack of the cachexia so characteristic of malignant tumor is one of the

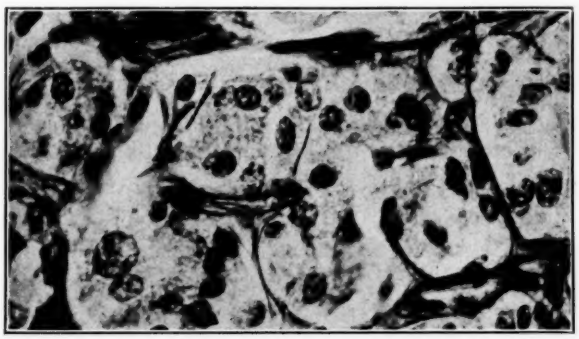

Fig. 9.—Eight mm. obj. c.c. 15 × camera 50 cm. × 600. Reduced. Case 5. J. H. The alveolar like arrangement of cells is noted.

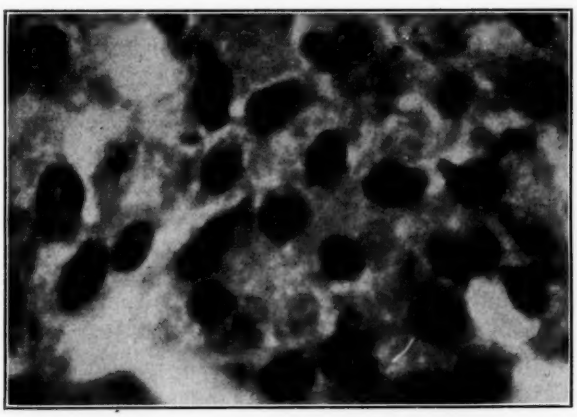

Fig. 10.—Four mm. obj. c.c. 15 × camera 50 cm. × 1200. Reduced. Case 5. J. H. Details of alveolus showing the characteristic endothelial type cell.

salient features of this neoplasm. Thus, we note that in one of our cases (C. S.) at the end of five years the patient is in good general condition. In another case (W. B.) with a history of somewhat over a year's duration, the patient is in excellent general condition. In a third (H. W.) twenty-five months after the appearance of the tumor, the patient shows no evidence of a generalized involvement and is in fairly good physical condition in consideration of the fact that at the present time he has a thyrotoxicosis which will have to receive attention before the tumor is dealt with. In Flynn's case, which was not treated surgically, death resulted two years and six months after the first nodule was noted.

The third important point in the clinical behavior of this tumor is the fact

that it is not affected in any way by radium or x-ray therapy. This feature has been noted in all five of our cases and in those reported by Flynn and Guttierrez. Quick and Cutler likewise note that the lack of response of endotheliomas to irradiation is an important feature in their diagnosis from a clinical standpoint.

We may note that in general the average age incidence of primary endothelioma of the lymph nodes appears to be somewhat earlier than that of carcinoma and is about the same as that for Hodgkin's disease and lymphosarcoma. There does not appear to be any particular sex distinction in the occurrence of this tumor. Obviously, no statement can be made from our observations regarding age or sex, as the patients who we observed are limited for the most part to veterans of the World War.

Despite the fact that the clinical features may be suggestive, the diagnosis is established by the pathologist after histologic examination.

Prognosis.—Endothelioma of the lymph nodes unquestionably offers a better prognosis as regards length of life than does sarcoma, carcinoma, or the average case of Hodgkin's diseases.

In one case of our series the patient came to the hospital in what was evidently a terminal stage of the condition, and death resulted after several months. Three other patients are living and well at the end of periods of one year, five years, and three and one-half years respectively. The fifth patient, the known duration of whose tumor is over fifteen months, shows no ill effects from endothelioma, but has at the present time a thyrotoxicosis.

We realize that the postoperative period in our patients is too brief to permit us to venture a statement as to whether or not the tumor has yielded to surgery. We will note with particular interest the case of (H) in whom at operation it was revealed that a small substernal portion of the growth could not be removed. Eight months, however, have elapsed since this operation, and at this time the patient is in excellent health with no evidence of recurrence. Zaffagnini and Lino each report cases, subjected to surgical operation and well at the time of the report. These reports like ours were made shortly after the operation.

The prognosis in individuals suffering from this tumor is favored by the fact that metastasis is rare and occurs late in the course of the disease.

So far none of the four patients in our series who are living show evidences of metastases. Our fifth one, in whom there was extensive involvement and in whom the tumor weighed 500 gm., at autopsy showed but one small metastasis, 1.5 cm. in diameter, in the left lung. In Flynn's case a metastasis to the left breast was noted. The extent of metastases in this case, however, cannot be known due to the lack of an autopsy.

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AN INSTANCE OF THYROID MEDICATION IN MERCURIAL NEPHROSIS*

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THE following is a report of a case of acute mercury poisoning which was successfully treated by thyroxin. It should be, we think, of particular interest to those who have to deal with heavy metal poisonings.

REPORT OF CASE

Mrs. E. C., aged twenty-eight, weight approximately 110 pounds, was admitted to the Jefferson Hospital on April 10, 1930, at 12:30 P. M. with the history of having inserted one tablet of bichloride of mercury 7½ gr. (0.49 gm.) in the vagina as an antiseptic. In the past she had used, she said, bichloride douches without harmful effects, and did not know that the use of the solid tablet without water would prove injurious. The tablet was placed in the vagina at 6 P. M. on April 5, one hundred and fourteen hours previous to admission to the hospital. Symptoms of poisoning were noticed within fifteen minutes. She had pain and burning in the vagina, felt faint, and weak, began to vomit and had urinary frequency. Her husband noticed a "poisonous" odor to her breath. The next day she had many bloody, watery stools, and developed a sore mouth. There were no abdominal cramps. After an initial diuresis, urinary function ceased at noon April 6, four days before admission. The family physician saw the patient twenty-four hours after the poison was taken. He instituted treatment at home, trying to force fluids, but the patient was unable to keep anything on her stomach. High sodium bicarbonate enemas were given. In the face of complete urinary suppression, persistent vomiting, and bloody diarrhea, the family physician thought it best to send her into the hospital.

The family history was unimportant.

The past history was essentially negative. There was no history of previous kidney trouble.

On admission the temperature was 100° F., the pulse 102, the respiration 20, the blood pressure 108 systolic and 60 diastolic. The patient was well nourished and appeared comfortable lying flat in bed. Sensorium was entirely clear. The physical examination was essentially neg-

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*From the medical service of the Jefferson Hospital.
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ative except for an extremely foul breath, white sloughy patches over the mucous membranes of the mouth and pharynx, a generalized abdominal tenderness, an inflamed and swollen vulva, and a necrotic vaginal mucosa. There was no generalized edema. Reflexes were normal. Anuria had been present for four days. Urine, 25 c.c. obtained by catheter, showed one-plus albumin, no sugar, casts, red or white blood cells. The hemoglobin was 90 per cent (Sahli), red cells 4,590,000, white cells 25,650 with increase of polymorphonuclear neutrophiles to 83 per cent.

TREATMENT AND COURSE IN THE HOSPITAL

The patient was seen too late even by the family physician to prevent absorption. Seven and one-half grains (0.49 gm.) of mercuric chloride had been placed in the vagina five days before admission to the hospital but no douches had been given. Conditions, it would seem, were almost ideal for complete absorption of the whole tablet. Surgery, we felt, had little to offer. Renal decapsulation certainly has not fulfilled expectations, and its results appear very doubtful. The question confronting us was, what treatment were we to use?

After absorption has taken place one would like, of course, to neutralize the toxin. Many chemical detoxicants have been tried and there are many conflicting reports as to their value. Zeigler's comment that recovery apparently takes place independently of the administration or the failure to administer any of the so-called specific chemical detoxicants seems well taken. In 1924, Dennie and McBride[1] stimulated a great revival of interest in chemical detoxication by emphasizing the success of sodium thiosulphate in the treatment of heavy metal poisoning. Careful consideration of their work, however, has led to skepticism in regard to their conclusions. Numerous men have found it ineffective in acute mercury poisoning. Haskell, Henderson and Hamilton,[2] for example, in experimental dogs poisoned by bichloride of mercury, found that sodium thiosulphate failed to prevent or even delay the effects of minimum lethal doses. Skepticism regarding the efficacy of sodium thiosulphate in mercury poisoning is, therefore, rather wide spread. It should never be used to the exclusion of the other well known and tried therapeutic measures.

In this case we adopted a method of treatment which seemed to combine the more effective measures of the various methods previously used. On the day of admission the patient was given 1,000 c.c. of 10 per cent glucose in 2 per cent sodium bicarbonate solution intravenously in divided doses of 500 c.c. each. Alkaline douches and enemas were ordered as local and eliminative measures. On the following day 400 c.c. of blood was withdrawn and 1,250 c.c. of 10 per cent glucose in 2 per cent sodium chloride solution given in divided doses. Alkaline diuretic mixture and milk were alternated every two hours by mouth. A sweat bath was given and hot packs were applied to the kidney regions. Magnesium sulphate was given by mouth and as rectal instillations. Eliminative and supportive treatment was adhered to very rigidly throughout the course of her illness. The intravenous salt and sugar solutions used, as well as the daily output of urine, are shown in Chart 1. Varying percentages were used in an effort to promote kidney action. Insulin was used along with the glucose from time to time. The alkaline therapy, sweats, and magnesium sulphate were continued. But the patient lost ground in spite of all treatment. The nonprotein nitrogen and creatinine were steadily rising and the urine output was not satisfactory. She became irrational, quite de-

lirious, restless, and had several hard convulsions. The outlook for the immediate future seemed very gloomy; in fact, every one felt that the case was hopeless.

On the basis of favorable reports from the use of thyroid extract in nephrosis and in view of the profound metabolic disturbance in mercurial intoxication, thyroid therapy, we felt, might offer some hope for her. On April 23, thyroid therapy was started. Thyroxin gr. 1/40 (1.6 mg.) was given subcutaneously in order to secure immediate effect. This dose was repeated on April 24 and 26. Desiccated thyroid gland was also given by mouth up to 6 gr. a day for seven days. One cannot be certain, though, as to how much of this was retained and absorbed, as the patient was vomiting a great deal. Signs of increased thyroid activity became evident. The pulse rate was more accelerated and definite exophthalmos developed. All

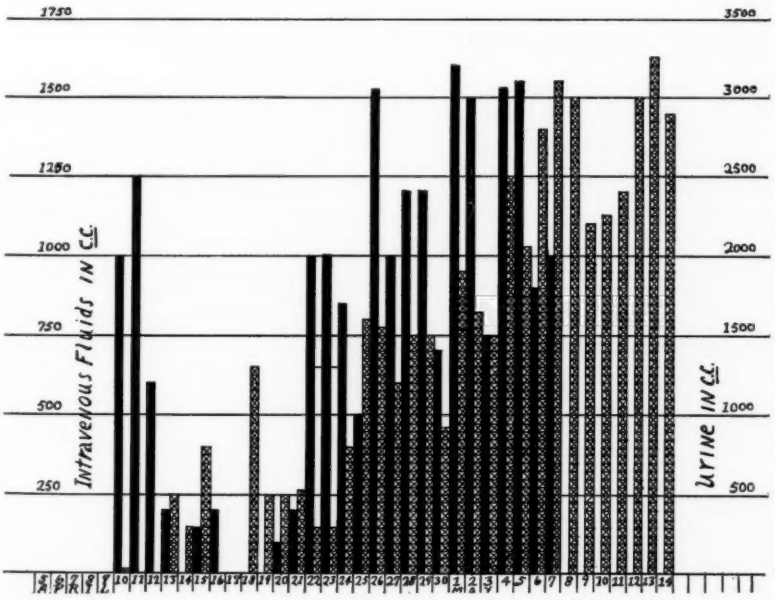

Chart 1.—Intravenous fluids (black area) and urine chart (shaded area). Glucose 10 per cent in Ringer's solution was given except on April 13, 15, 16, 20, and 21, when equal parts of glucose 50 per cent and sodium chloride 30 per cent, were used.

the other therapeutic measures that had been used previously were continued. On the second and third days after instituting thyroid therapy, the urinary output increased and continued until recovery became apparent and certain. Definite clinical improvement was evident within three days. Indeed, the whole picture changed from a seemingly hopeless one to an encouraging one, and the patient made a rapid recovery.

Laboratory Data.—The specific gravity of the urine was low throughout, ranging from 1.001 to 1.011. The albumin was heavy at first and gradually decreased in amount, there being only a trace on discharge. It is interesting that no casts were ever found. There was a trace of blood microscopically in a few of the voided specimens and a fairly constant trace of pus. Epithelial cells were present in great numbers. There was sugar in the urine at times, probably due to the therapeutic measures. No acetone bodies were found and the reaction of the urine was

usually alkaline. There was no excretion of phenolsulphonephthalein on May 7, nor on May 12, but a trace in the two hours on May 19. Four months later there was 70 per cent excretion of the dye in a two hour period. Urine examination at this time was negative.

The blood chemistry was interesting. Daily determinations were made of nonprotein nitrogen, creatinine, and chlorides. The nonprotein nitrogen reached 288 mg. per 100 c.c. of blood and the creatinine 10 mg. per 100 c.c. of blood. The two curves ran parallel and each reached its maximum on the same day. These figures would, no doubt, have been much higher had not large quantities of intravenous fluids been given. The blood chlorides were low on admission, being only 265 mg. per 100 c.c. of blood. The saline intravenously raised the level to about 400 mg. and it kept around this point.

The blood picture changed markedly during the course of her illness. A severe secondary anemia developed. The hemoglobin went down to 31 per cent (Sahli), red cells 1,910,000 on May 21. The hemoglobin was 53 per cent with 2,900,000 red cells on May 9 at a time when the patient already showed marked clinical improvement. It is interesting that the anemia progressed so far after every other sign of improvement became evident. As stated above, there was a marked leucocytosis of 25,650 with an increase of the polymorphonuclear neutrophiles to 83 per cent. Weiss[3] states that the degree of elevation of the white count corresponds closely with the severity of the symptoms of intoxication. This leucocytosis, he says too, may reach 20,000 and then decline, or remain elevated and carry an unfavorable prognosis. But in this particular case the leucocyte count was not followed closely. The blood picture improved rapidly on liver extract and iron. The basal metabolism was plus 2 on discharge, May 25, 1931.

DISCUSSION

The intravenous use of a hypertonic salt and glucose solution, consisting of equal parts of 50 per cent glucose (solution) and 30 per cent salt (solution), caused a moderate diuresis. But this did not persist. Fairly large quantities of a 10 per cent glucose solution failed to produce a diuresis in the beginning. However, intravenous fluids were continued chiefly in the form of 10 per cent glucose in Ringer's solution. The bicarbonate glucose solution and insulin lessened the acid intoxication which has been found to be constantly present in acute mercuric chloride poisoning. The beneficial effect of this is obvious, as delayed kidney injury is associated with the development of this acid condition.[4] A hypochloremia is constantly present in acute bichloride intoxication caused by the persistent vomiting and loss of fluids by bowel.[5] The saline and Ringer's solution raised and maintained the blood chlorides at a satisfactory level. The large quantities of intravenous fluids undoubtedly held the concentration of the nonprotein nitrogenous substances at a lower level. This may be explained by the factor of dilution, by the diuresis produced, and by the ability of carbohydrates to spare body proteins.

Mercury is excreted largely by the bowel and to a less extent by the kidneys, skin and other mucous surfaces. Magnesium sulphate by mouth and by rectum seems an important eliminative measure. The value of the sweat bath is hard to estimate. Lambert and Patterson[6] attached a great deal of importance to it.

Thyroid medication appeared to be very definitely a life saving measure in this case. In regard to its beneficial effect nothing definite can be said concerning its mode of action. However, the pronounced increased urinary output immediately following the administration of thyroxin suggests strongly that the thyroxin may well have been a factor in hastening the return of renal function. Further studies along this line, then, would seem desirable. During the course of treatment with thyroxin and thyroid extract the interesting observation was made of the experimental production of bilateral exophthalmos.

The phenolsulphonephthalein elimination in cases of acute bichloride poisoning presents a most interesting problem. It has been observed that the dye fails to be eliminated during the acute phase of the disease and during early convalescence. But within a relatively short period of time after recovery is assured, the kidneys eliminate normal amounts of the dye in the absence of preexisting nephritis. In the case under discussion there was no elimination of the dye on May 7, nor on May 12, and barely a trace in all specimens on May 19, five days before her discharge. Four months later there was normal phthalein elimination, 70 per cent in two hours. This case demonstrates clearly that this test is not a reliable index of kidney function during the recovery phase of acute bichloride poisoning.

In consideration of the facts observed in this case and from the histopathologic picture in the acute bichloride kidney, one may conjecture as to the most probable mechanism of phthalein excretion in the human kidney. It has been shown that the glomerular units are histologically intact and appear normal while the tubules show most extensive necrosis of the epithelium. Such cases furnish beautiful examples, it seems to us, of localized injury with respect to the kidney. It would seem, too, that much could be learned about the function of the various units by careful study of these cases. If the glomeruli were concerned particularly with the phthalein elimination, one would expect its appearance in the urine unless reabsorption were complete in the tubules; or unless no free phthalein existed in the blood, it being in a bound and nonfiltrable form. The first of these assumptions is unlikely and the latter has been demonstrated untrue.[7] Since there is no detectable dye in the urine, the data at hand points, in our opinion, to the tubules as the main seat of phthalein elimination in the human kidney, as Marshall[8] and his collaborators maintain.

Acknowledgment is made to Dr. Lawson for the privilege of reporting this case on whose service it was treated.

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OUTLINE OF A METHOD FOR THE DETERMINATION OF THE STRENGTH OF THE SKIN CAPILLARIES AND THE INDIRECT ESTIMATION OF THE INDIVIDUAL VITAMIN C STANDARD*†

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THE strength of the skin capillaries can be systematically ascertained by a test which is an extensive elaboration of the clinical capillary test used by C. Leede,[1, 2] Alfred F. Hess,[3, 4] R. Stephan,[6, 7] and others. The following modifications had to be made in the clinical capillary test to transform it into a test of the strength of the capillaries, satisfactory from the physical point of view:

The veins of the upper arm must be subjected to manometrically measured pressure, and in most cases *more than one degree of pressure* must be applied to make the determination. Each degree of pressure applied should be lower than the diastolic blood pressure in the brachial artery of the subject of examination. Otherwise the afflux of arterial blood is also partly obstructed. Finally, the skin area within which the observations are to be made should be definitely limited as to position, shape, and size.

In the author's method, pressures of 35 and 50 mm. Hg were selected. The skin area to be examined is circular, with a diameter of 60 mm., and its center coincides with the center of the hollow of the elbow. In order to obtain sufficient sensitiveness in this test, it is necessary to maintain the pressure for fifteen minutes. In carrying out this test it proved advisable to fix limits between the various grades of strength in the following manner, according to a descending scale:

Grade I. No petechiae within the examined skin area at a pressure of 50 mm. Hg in fifteen minutes.

Grade II. Petechiae appear at a pressure of 50 mm. Hg, but their number does not exceed 6.

Grade III. More than 6 petechiae appear at 50 mm. Hg, but none or at most one at 35.

Grade IV. At least 2 petechiae are present at a pressure of 35 mm. Hg.

As we know, A. F. Hess[3] found that his "capillary resistance test" is positive in scurvy and he also states that it forms a clue in the diagnosis of latent scurvy, a conclusion which is confirmed in a paper of H. Öhnell.[8] But where Hess applied the test only in cases of scurvy, manifest or latent, the author has gone a step further and inquired into the possibility, by means of this test, of revealing much milder deficiencies in vitamin C, viz., such as lie between the smallest discernible deviation from a normal vitamin C level and the highest deviation possible without giving rise to actual symptoms of disease. The latter class together with cases of latent and manifest scurvy form a complex to which the author suggests giving the general name of *vitamin C undernourishment*.

The author determined the serviceability of the capillary strength test as a criterion of even low degrees of vitamin C undernourishment by the following process:

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†As to the results already arrived at by this method, more detailed information is available in the author's paper. "A Method of Establishing the Vitamin C Standard and Requirements of Physically Healthy Individuals by Testing the Strength of Their Cutaneous Capillaries," Skandinavisches Arch. f. Physiol. **61**: 225-270, 1931.

Two physically healthy adult persons were placed on a scorbutic diet which was supplemented by precisely measured daily rations of juice freshly squeezed from yellow Mediterranean oranges, and these daily rations were altered by progressive steps in the course of the investigation. It was ascertained (in January and February, 1930) that the original normal strength of the skin capillaries of the subjects of experiment fell to an abnormally low level when the ration of antiscorbutic juice was kept low for weeks, but that this change in the capillary strength was reversible, the strength returning gradually to normal when a sufficient amount of juice was given for a sufficiently long time (several weeks).

By the testing of the capillary strength both of healthy vegetarians and of school children who had been suffering from vitamin C undernourishment, but whose vitamin C standard was raised by the eating of oranges (after which new capillary strength tests were made), the grades of capillary strength corresponding respectively to a normal and a subnormal vitamin C standard were ascertained. By this investigation, the capillary strength test was actually elaborated into *a nutritional hygiene test by means of which, in a great many cases, individual vitamin C standards can at any time be indirectly ascertained.*

A determination of the strength of the skin capillaries of an individual may, of course, be made whether he is healthy or not. In general, however, such a determination does not offer a solid foundation for conclusions as to the vitamin C standard unless the person examined is entirely healthy with the sole exception of possible symptoms of lowering of his vitamin C standard. Nevertheless, in some not unimportant diseases ("Points to be noted," No. 3, 4 and 7), conclusions as to the vitamin C standard seem to be justified, although the conditions just mentioned are not fulfilled.

I. DESCRIPTION OF APPARATUS

The principal instruments for ascertaining the capillary strength are the following (Fig. 1):*

1. *A rubber stamp* (*a*) and ink pad for imprinting on the skin of the hollow of the elbow a colored ring within which the capillary observations are to be made. The ring is stamped so that its center coincides with that of the elbow. For adults and children down to two years old, a stamp making a ring 60 mm. in diameter is used, and for younger children, one making a ring 40 mm. in diameter. If the small stamp is used, the corresponding number of petechiae in a circular area measuring 60 mm. in diameter can be computed by multiplying by 2.25.

2. A large Politzer *rubber syringe* (*b*), with an air-tight connection with its nozzle.

3. A *screw compressor* (*c*), so constructed that when its compressing surfaces of flat metal are farthest apart, the fully distended rubber syringe will fit in between them without distortion, while turning of the screw as far as it will go will completely compress the syringe.

4. A *rubber tourniquet* (*d*), of the kind used in measuring the blood pressure, for producing an external pressure on the veins of the arm. For adults and children down to the age of about ten, the rubber part of the tourniquet should be 12 cm. wide, for children between nine and five, 8 cm., and for still smaller children,

*The complete apparatus for the test of capillary strength herein described may be obtained from Kirurgiska Instrument Fabriksaktiebolaget, Stockholm, Sweden.

5 cm. The canvas part of the tourniquet may not extend over the edge of the rubber part for more than a few millimeters.

5. A *low mercury manometer* (*m*) with conspicuous marks on the scale at 35, 50, and 65 mm. Hg.

6. A Y-shaped metal tube and air-tight, flexible rubber tubes, by means of which the air chamber of the tourniquet, the syringe in the compressor and the inlet tube of the manometer may be joined in a single closed air-system.

7. A *magnifying lens* (*e*) of 5 D with which to inspect the marked-off skin area for possible petechiae.

8. A circular *plane glass* (*h*) set in a metal rim with a handle, for locally pressing the blood out of the skin. By this, it is possible in cases of doubt to distinguish between merely hyperemic red spots and genuine petechiae. Hyperemic spots will disappear on pressure, whereas petechiae will become more distinct.

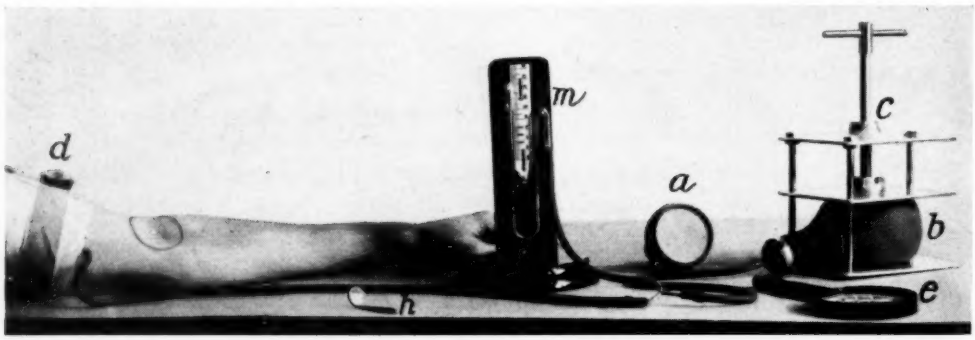

Fig. 1.

II. FURTHER REQUIREMENTS FOR MAKING THE TEST

1. In order to place the arm on a table in the plane of the heart, either an adjustable chair with back support, or a suitable number of wooden boards.

2. It is convenient to employ a block of wood for raising the manometer above the level of the table, thus facilitating the reading of the mercury meniscus. When the apparatus is set up for use, the tubes of the manometer should be strictly vertical.

3. A watch with a second hand or preferably a stop-watch.

4. Glass tubes with capillary points; black ink to indicate the petechiae and green ink for other spots with which the petechiae might otherwise be confused, and for any petechiae which in exceptional cases may be present before the application of pressure.

III. HOW TO MAKE THE CAPILLARY STRENGTH TEST

During the test, the skin area to be inspected and consequently the surrounding air as well should have the proper temperature. It has been found that an increase in the temperature of the skin (causing dilatation of the vessels) tends to increase the number of petechiae, while a lowered temperature (causing contraction of the vessels) tends to decrease the number of petechiae. In cases of more pronounced deviation from the normal temperature of the skin, therefore, the results

obtained by this method will not be reliable. In view of this influence of temperature, the following precautions are necessary:

1. The temperature of the room should be between 16° C. and 21° C.

2. The subject must not have had a hot bath or a steam bath on the day of examination, nor have taken part in any sports, nor, within the three hours immediately preceding the examination, done any gymnastic exercises.

3. In the winter, if the outdoor temperature is low, the subject must have been indoors in the required temperature sufficiently long not to feel in the least cold.

The test should be carried out in broad daylight or in *exactly equivalent* artificial light, e. g., that of a daylight lamp of sufficient strength. (The petechiae are even more distinct in green light than in daylight.)

The subject is placed in a comfortable chair with support for his back. (Examination in the recumbent position is also practicable, but would probably seldom be chosen.) One of the arms is bared to the armpit, care being taken that the rolled sleeves exert no pressure on the arm. Any garment doing so should be removed. The arm is then placed horizontally *in the plane of the heart.* This precaution is very important if a reliable result is to be obtained. If it is neglected, the absolute pressure in the capillaries tested may vary even though the manometer records the same level of pressure. If, for instance, the arm is placed 10 cm. below the level of the heart, there will be besides the pressure registered on the manometer, an additional hydrostatic pressure of $\frac{100 \times 1.058}{13.6}$, equal to 7.8 mm. Hg, and this pressure will also affect the capillaries during the test.

With the subject sitting down, the arm is most accurately placed when the surface to be examined is in the plane of the insertion of the third rib into the sternum. This can be done either by placing the arm directly on the table and adjusting the trunk by means of a swivel chair, or by supporting the arm at the desired level above the table on top of a suitable number of boards.

During the entire test, the subject must keep his arm as well as the rest of his body perfectly quiet.

A colored ring is imprinted with the rubber stamp on the arm with its center coinciding with the center of the hollow of the elbow. The area of the skin thus outlined must be carefully and thoroughly inspected through the lens before pressure is exerted. If one or more formations which may later be mistaken for fresh petechiae (such as small areas of hyperemia, capillary ectasiae, or remains of petechiae from previous tests) are visible, they should be carefully indicated, most suitably by green ink, before the test is made.

The rubber tourniquet is then wound tightly around the arm and fixed, care being taken that *the lower edge of the tourniquet is at least 2.5 cm. above the nearest part of the colored ring.* Petechiae often appear in greater numbers just below the lower edge of the tourniquet rather than further away, and the distance between should therefore not be too small.

The compressor screw should be turned *as quickly as possible,* the pressure in the tourniquet and consequently the external pressure on the veins of the upper arm being thereby raised to the desired level (35, 50, or 65 mm. Hg). At the moment the mercury meniscus in the manometer reaches the proper level, the time is

taken or the stop-watch started. The pressure is then kept constant for fifteen minutes, if necessary by adjustment of the compressor.

When the pressure is released, nothing should be done until the cyanosis of the arm has disappeared; this may be hastened by the raising of the arm for fifteen seconds. *The petechiae should be counted within half an hour after the release of pressure.* Within an hour, some of the petechiae may already have disappeared. When the number of petechiae in the ring exceeds 3, it is advisable to indicate them by making a black ink spot close to each petechia, after which the spots are counted. If the number is very great, it is best to divide the circle into quadrants by two lines passing through the center at right angles to each other; the petechiae in only one of these quadrants need then be counted and multiplied by 4.

IV. CONCLUSIONS FROM THE CAPILLARY STRENGTH TEST

In order to ascertain by the method in question to which grade of strength the capillaries of a person belong, we proceed in the following manner:

One of the arms is subjected to a pressure of 50 mm. for fifteen minutes. If no petechiae appear, the capillary strength is of Grade I. If a pressure of 50 mm. gives up to 6 petechiae, it is of Grade II.* If a pressure of 50 mm. produces more than 6 petechiae, a new test should be made *on the other arm* with a pressure of 35 mm. If no petechiae, or not more than one appears, the capillary strength is of Grade III. If two or more petechiae appear, it is of Grade IV.

The relation between the strength of the skin capillaries of a healthy person determined in this manner and his vitamin C standard at the time of the test has been experimentally ascertained for *the Nordic race* only (cf. G. F. Göthlin[5]). In 1930, the Hygienic Section of the League of Nations was asked to take the initiative in an investigation of this matter along the same lines as in the paper cited in the case of other races living in temperate climates for whom such an investigation might be valuable.

In my investigation the following was found to be true of healthy members of the Nordic race:

If the technic outlined is adhered to and *a pressure of 50 mm. for fifteen minutes does not produce more than 4 petechiae in the encircled area, we may conclude that the vitamin C standard is normal.*

If 50 mm. Hg produces more than 8 petechiae, or if 35 mm. Hg produces more than one petechia, the vitamin C standard is definitely inferior.

Since by this method it is not possible to draw definite conclusions as to the vitamin C standard from differences of less than 4 in the number of petechiae within the outlined area at a pressure of 50 mm. cases with from 5 to 8 petechiae are regarded as *transitional*, 5 or 6 petechiae rather suggesting a normal standard, and 7 or 8 a subnormal one, though owing to the limits of error involved by the method, definite conclusions cannot be drawn.

V. POINTS TO BE NOTED

1. In investigations, on a large scale (as in schools, regiments, and the like), into individual vitamin C standards or average standards on certain diets, a good deal of time can be saved by connecting the tourniquets of three persons at a time

*In the examination of members of the *Nordic race*, it is very unusual for more than one petechia to appear at 35 mm. Hg in a case in which there are only 6 or less at 50 mm. Hg.

to one and the same manometer and compressor. For this purpose a tube with 6 branches is substituted for the Y-tube. To the sixth branch is coupled an air blowing pump (like those used in blood pressure determination).

When the pressure has been raised by the aid of this pump almost to the required level, the pump is shut off from the air system by the application of a clamp on its rubber tube. By means of the compressor the pressure is exactly adjusted and maintained. In this way, three people are subjected simultaneously to the fifteen-minute period of compression.

2. If a series of tests on one person is desired, it is necessary to allow at least a fortnight, but preferably a month, to intervene between each test so that the ruptured vessels may have time to heal perfectly. By the testing of alternate arms, it is possible to have a month intervening between tests on the same arm and yet have new tests every fortnight. More frequent tests than this are hardly worth while.

In such series of tests, a careful inspection of the skin before the test, is of especially great importance. When only one arm is tested, the 35 mm. pressure should be applied *before* that of 50 mm., and it is desirable that at least an hour be allowed to elapse between these two tests.

3. It is remarkable that simple arteriosclerosis does not by itself reduce the strength of the skin capillaries, at least to judge from the observations hitherto made on a small scale (10 cases). This method apparently can therefore also be used to determine the vitamin C standard of persons suffering from simple arteriosclerosis, but otherwise healthy.

4. Since there may be cases of latent tuberculosis among those tested in a mass investigation, it is interesting to note that tests made on a small number (12 cases) of persons with manifest pulmonary tuberculosis do not support any assumption that tuberculosis alone reduces the capillary strength.

5. In various acute infectious diseases (such as measles and scarlet fever), a reduction in the strength of the skin capillaries is found[1, 2, 6, 7] and may persist in many cases for a rather long time, but the cause of this reduction has not yet been investigated. In the case of persons who have within the two months immediately preceding the investigation suffered from an acute infectious disease, it is therefore, at the present stage of our knowledge, wisest not to estimate the vitamin C standard from the strength of their capillaries.

6. No such estimate can be made in albuminuric conditions either, since these appear generally to reduce the strength of the skin capillaries.

7. In cases of achylia and uncomplicated afebrile stomach ulcers the vitamin C standard may be estimated from the results of the capillary strength test.

When inferior capillary strength is found in persons suffering from achylia or chronic enteritis, it should be kept in mind that part of the vitamin C in the food may be lost; in the former cases through the unfavorable conditions for the preservation of that vitamin in the digestive canal (alkaline reaction), and in the latter because of the difficulties in resorption of the vitamin, which are presented by the catarrhal alteration of the mucous membrane.

SUMMARY

1. A test is described which makes it possible to determine the degree of strength of the skin capillaries in the hollow of the elbow.

2. The capillary strength, ascertained by this method, indirectly reveals the vitamin C standard of persons who are quite healthy or exhibit only such deviations from health as are in themselves due to a low vitamin C standard.

3. It is suggested that with this same method it is probably also possible to estimate the individual vitamin C standard in cases of uncomplicated arteriosclerosis, afebrile tuberculosis, achylia, and uncomplicated afebrile stomach ulcers.

4. This method may be used as a test of the individual vitamin C standard by physicians, hygienists, and dentists in their practice. It can also be used in the statistic examination of groups (e. g., in the army, in boarding schools, in orphanages, in old people's homes, in asylums, and in prisons) in order to ascertain whether the diets in use provide a sufficient supply of vitamin C.

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TWO CASES OF ANEMIA OF PREGNANCY WITH THE CLINICAL FINDINGS OF MYASTHENIA GRAVIS*

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A LARGE number of cases of pernicious anemia of pregnancy have been reported in the literature and the symptoms, signs, and blood findings have been clearly stated by Schmidt,[1] Larrabee,[2] Allan,[3] Rowland,[4] McSwinney,[5] Balfour,[6] and others. The signs and symptoms most frequently present are: weakness, breathlessness, palpitation, pallor, and gastrointestinal disturbances such as nausea, vomiting, and diarrhea. There is usually a history of fever or its presence during the course of the disease. These signs and symptoms may be accompanied by a blood picture of a primary (Schmidt,[1] Balfour,[6] etc.), hemolytic (Minot,[7] Rowland,[4] etc.), secondary (Osler,[8] Evans,[9] Mills,[10] etc.), or aplastic (Larrabee[2]) type of anemia. The pallor is usually marked and icterus may or may not be present depending upon the type of anemia.

The percentage of cases in which the liver and spleen have been found enlarged has varied in the different series of cases reported. In Balfour's[6] series of 150 cases

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the liver was enlarged in 8 per cent and the spleen in 18 per cent, while in 10 cases reported by Larrabee[2] the liver was enlarged in 16 per cent and the spleen in 70 per cent. The degree of asthenia has also varied. Wills and Mehta[11] in India remark

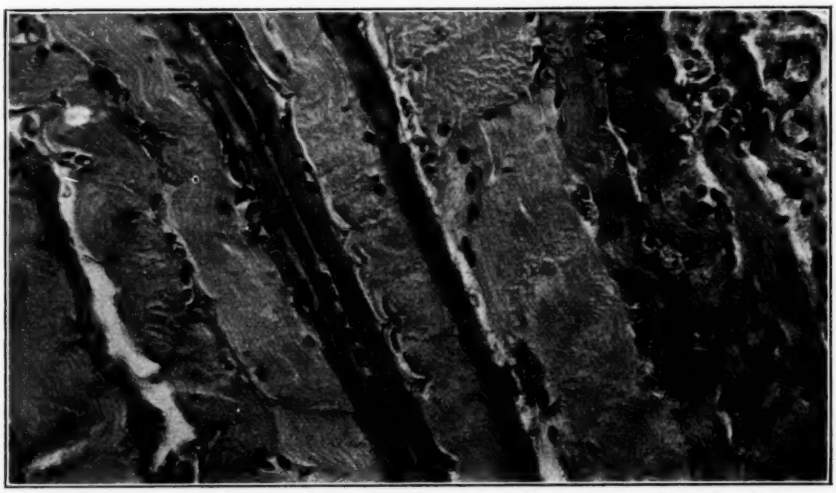

Fig. 1.—A photomicrograph of a section of the gastrocnemius muscle obtained at biopsy from Case 1. There is a marked variation in the size of the muscle fibers, the larger ones appear granular while the small fibers have a hyaline or homogenous appearance. In both, the cross striations are absent. In the intermediate sized fibers the cross striations are present in some areas. The blood vessels appear normal and there is no perivascular infiltration. Magnified 300 diameters.

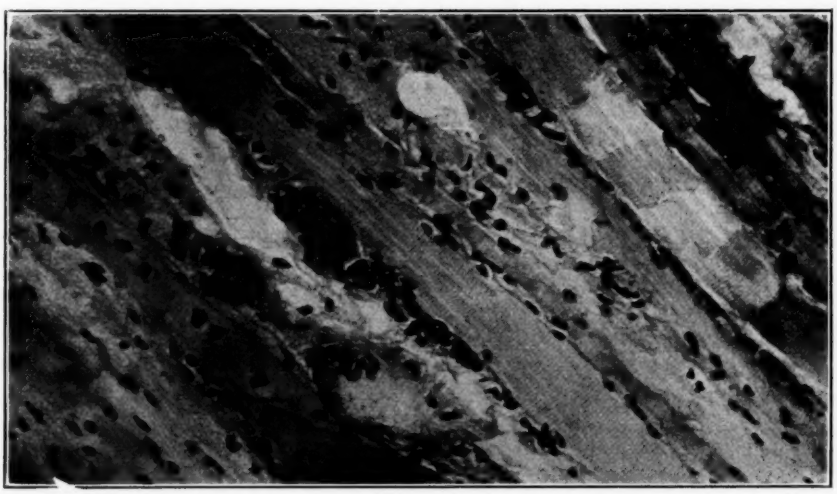

Fig. 2.—A photomicrograph of a section of the gastrocnemius muscle obtained at biopsy from Case 1. The variation in the size of the muscle fibers is less marked than in Fig. 1 and the cross striations are more prominent. Areas of localized hyalinization of the muscle fibers are present. In scattered areas throughout the sections, it appears that the sarcoplasm was being absorbed and the sarcolema with pyknotic nuclei remains in a retracted state that gives the appearance, at first glance, of a collection of cells that may be mistaken for plasma cells or lymphocytes. Magnified 300 diameters.

upon the activeness of many of their patients, while Schmidt[1] says that sooner or later asthenia becomes the chief complaint.

Two patients with anemia of pregnancy entered the Indianapolis City Hos-

pital at approximately the same time with the diagnosis of toxemia of pregnancy, and in both cases the asthenia was of such marked degree that it suggested myasthenia gravis. Burr and McCarthy,[12] in 1901, reported two cases of myasthenia gravis. In one case the symptoms of weakness and numbness began one year after an abortion, progressed during the next year, and terminated fatally two years after onset. At necropsy a three or four months' fetus was discovered in the uterus. Blood studies were not reported, however, it was noted on admission that the patient was somewhat pale. Hun, Blumer, and Streeter[13] collected from the literature nine cases of myasthenia gravis associated with pregnancy.

CASE 1.—M. G., a white married female, aged thirty-one years, entered the hospital in a semi-stuporous condition. She had enjoyed good health until the present illness. She had had two normal pregnancies, the last one three years before. The last menstruation occurred three months before admission.

The present illness began seven weeks before admission with nausea and vomiting after each meal. Vertigo, headache, and blurring of vision developed. There was difficulty in focusing the eyes for any length of time. One week before admission weakness became so marked that she was unable to phonate and at times appeared irrational.

On physical examination the patient appeared toxic, was irrational and easily excited. There was a slow lateral mystagmus of both eyes with ptosis of the left eyelid and paralysis of the left occipitofrontal muscle. Dyspnea was marked while lying quietly, and the heart rate was 140 beats per minute. The axillary temperature was 99° and the blood pressure was 122/80. There was an enlargement of the uterus equivalent to four months' pregnancy. The liver and spleen were not enlarged. The muscles were flaccid and atrophied with marked coarse tremors and tenderness upon deep palpation. The knee jerks were absent. The urine examination revealed several white blood cells and a trace of albumin. A secondary anemia was present with R.B.C. 3.3 million, Hb. 57 per cent, and 5.1 per cent reticulocytes. The W.B.C. was 12,000. The blood, nonprotein nitrogen, sugar, calcium, bilirubin, and Wassermann were normal. The spinal fluid was negative.

A diagnosis of toxemia of pregnancy was made and pregnancy was terminated one week after admission. The clinical condition remained unchanged during the next two months. During the third month in the hospital she was able to phonate, the pulse rate and temperature declined, and there was marked improvement in muscular weakness, but she continued to have irrational periods.

Electrical reactions of the muscles showed a decreased response and marked fatigability to intermittent faradic current. Galvanic current showed no evidence of peripheral nerve lesion.

A specimen of gastrocnemius muscle obtained at biopsy showed considerable atrophy of the muscle fibers without definite lymphorrhagia as shown in Figs. 1 and 2.

The patient was discharged markedly improved after 132 days in the hospital.

CASE 2.—E. I., a colored married female, aged twenty-five years, entered the hospital in a stuporous condition. She had always been well until the present illness. She had one pregnancy that ended in stillbirth at nine months. The last menstruation was four months before admission.

The present illness began ten weeks before admission with morning nausea and roaring in the head. Vomiting and weakness developed and four days before entry she became drowsy with loss of orientation and coordination.

On physical examination the patient did not appear toxic. There was a lateral mystagmus of both eyes. The voice was weak and dyspnea was present while lying quietly. The heart rate was 100 beats per minute. Temperature 101°. The blood pressure was 95/33. There was an enlargement of the uterus equivalent to four months' pregnancy. The liver and spleen were not enlarged. The muscles of the extremities were flaccid. The knee jerks and biceps reflexes were absent while the other reflexes were normal.

The urine showed a trace of albumin and 50 W.B.C., 10 R.B.C., and an occasional hyaline cast per high power field. A primary type of anemia was present with R.B.C. 2.9 million, Hb. 65 per cent, 6.8 per cent reticulocytes, and W.B.C. 8,800. The blood, nonprotein nitrogen, sugar, calcium, bilirubin, and Wassermann were normal. The spinal fluid was negative.

The diagnosis of toxemia of pregnancy was made and treatment instituted. Two months after entry the clinical condition remained unchanged. Gastric analysis showed free HCl present. Electrical reactions of the muscles showed marked fatigability to intermittent faradic current and no evidence of peripheral nerve lesion by galvanic current. A section of the gastrocnemius muscle revealed atrophy of the muscle fibers without lymphorrhagia as shown in Figs. 3 and 4.

Fig. 3.—A photomicrograph of a section of gastrocnemius muscle obtained at biopsy from Case 2. There is a marked variation in the size of the muscle fibers and the nuclei of the sarcolema appear more prominent about the small fibers. The areas of retraction of the sarcolema, with the collection of its pyknotic nuclei, as shown in Fig. 2, are less numerous in the sections of this case and are not shown in the illustrations. Magnified 300 diameters.

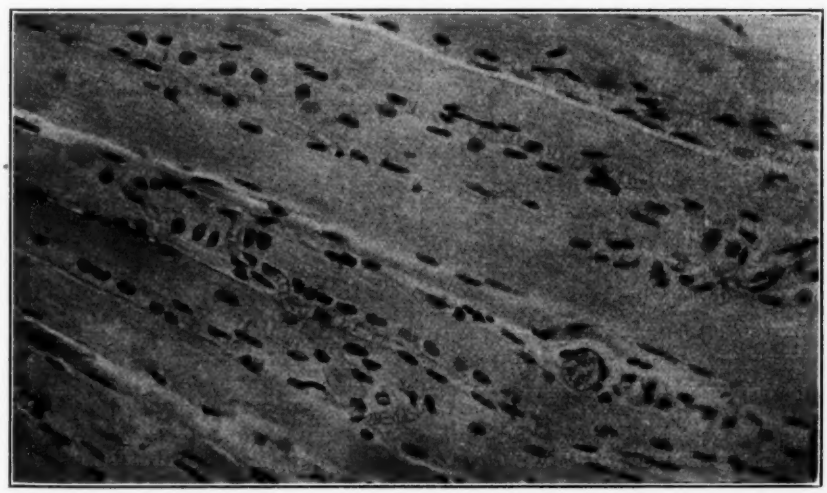

Fig. 4.—A photomicrograph of a section of the gastrocnemius muscle from Case 2. The variation in size of the muscle fibers is less marked and the cross striations are present in some areas. The blood vessels appear normal without perivascular infiltration. Magnified 300 diameters.

There was steady improvement until discharge 113 days after admission.

Two months later she had a normal delivery. On the eighth day postpartum dysuria and fever developed. There was a sudden return of the previous symptoms and the patient reentered the hospital.

The blood showed R.B.C. 1.0 million, Hb. 21 per cent, reticulocytes 2.5 per cent, and W.B.C.

6,900. The other significant physical and laboratory findings were similar to the previous admission.

Three vials daily of liver extract, No. 343, were begun. The reticulocyte and red blood cell

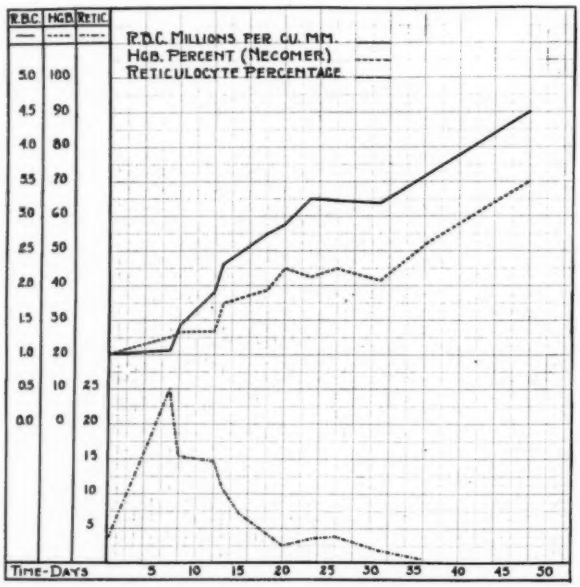

Fig. 5.—(Case 2) Shows response of blood to 3 vials daily of liver extract, No. 343.

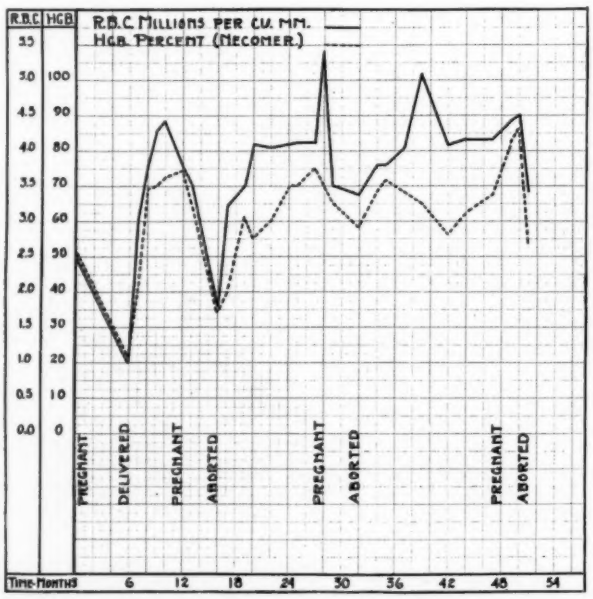

Fig. 6.—(Case 2) Shows the course of the blood during four years' observation with four pregnancies.

response are shown in Fig. 5. There was steady clinical improvement until discharge, seventy-five days after admission.

During the next three and one-half years there were three pregnancies, each terminated at four or five months. Anemia was present during each of these pregnancies as shown in Fig. 6.

The previous symptoms were present during the first and last of these pregnancies but absent during the second.

Electrical reactions of the muscles during the last pregnancy were normal and a specimen of muscle obtained at the site of the previous biopsy showed no atrophy or lymphorrhagia.

Case 1 moved from the city soon after discharge and was not followed, while Case 2 was followed for four years. Both cases presented the typical clinical picture of myasthenia gravis with the myasthenic reaction but without the presence of lymphorrhagia in a skeletal muscle. The myasthenic reaction has been found in numerous conditions but lymphorrhagia in a skeletal muscle has been reported only in myasthenia gravis and in one case of amyatrophic lateral sclerosis, according to Keschner and Strauss,[14] and lymphorrhagia is found in only about 50 per cent of the cases of myasthenia gravis. In myasthenia gravis the presence of lymphorrhagia is not parallel to the severity of the involvement of a muscle, and has not always been demonstrated in muscles with marked involvement.

In Case 2 there was marked atrophy of the muscle fibers in the specimen removed at the first biopsy, while in the specimen obtained four years later from the same muscle and at the site of the previous biopsy, the histologic picture was normal.

The subsequent findings in Case 2 are in accord with the diagnosis of pernicious anemia of pregnancy, although Case 1 had a secondary type of anemia. The presence of a secondary anemia with the clinical picture of pernicious anemia of pregnancy has been reported in two cases by Evans. Wills and Mehta have seen patients with secondary anemia associated with pregnancy rapidly change to a primary type. The presence of fever in both cases is more in accord with this diagnosis than with the diagnosis of true myasthenia gravis.

The parallel progress of both patients even though pregnancy was terminated in Case 1 and not in Case 2 agrees with the statement of Schmidt that premature termination of pregnancy, induced or otherwise, did not have any influence upon the progress or improvement of the clinical condition of the patient.

The electrical reactions in pernicious anemia of pregnancy have not been reported in the literature. The pathologic findings were reviewed by Schmidt, and Balfour reported the findings in two cases, but the histologic findings in the skeletal muscles were not stated.

The presence of primary pernicious anemia in the negro is rare, only 30 cases have been reported in literature according to Mathews.[15] In Case 2, the anemia was of a primary type and occurred in a negro with free HCl present in the gastric contents. We have been unable to find any report in the literature of pernicious anemia of pregnancy in the negro.

In Case 2, the anemia and symptoms returned immediately after delivery and again in varying degrees during the three subsequent pregnancies, as shown in Fig. 5. This is in agreement with the findings of Murdock, Evans, and McSwinney. The premature termination of the three subsequent pregnancies further supports McSwinney's findings that premature termination of pregnancy tends to occur.

The response of the blood, in Case 2, following the administration of liver extract, is shown in Fig. 6. The efficiency of liver and liver extract upon the anemia in the pernicious anemia of pregnancy has been shown by Evans,[9] Peterson, Field and Morgan,[17] and others. However, the effect of liver extract upon the recurrence

of the symptoms in subsequent pregnancies and upon the tendency to premature termination of pregnancy has not been mentioned in the literature and further study is needed in this regard.

SUMMARY

1. Two cases of anemia of pregnancy with the clinical findings and electrical reactions of myasthenia gravis are reported, one patient having a secondary and the other a primary type of anemia.

2. The primary type of anemia occurred in a negro patient, with free HCl present in the gastric contents, and a typical response of the blood was obtained following the administration of liver extract, No. 343.

3. The case with a primary type of anemia has been followed for four years with three subsequent pregnancies, all of which were accompanied by a relapse of the blood.

4. The clinical findings and electrical responses of myasthenia gravis were absent in the negro patient when examined four years later.

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A CASE OF SARCOMA BOTRYOIDES*

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SARCOMA botryoides is the name applied to a comparatively rare polypoid tumor occurring within the cervix uteri. The unusual character of this tumor renders it a well defined pathologic entity. According to Wilms,[1] the sarcomas botryoides are true mixed tumors developed from misplaced embryonal mesodermic cells. Their early appearance and complex histologic structure indicate their origin to be probably the result of some embryogenic disturbance.

Fig. 1.—Thick edematous mucous membrane of bladder and long urethra.

A review of the literature shows a marked discrepancy in the number of cases reported. McFarland[2] collected 34 cases, Veit[3] 40, Labhardt[4] 60, and K. Adler[5] could find only 41 authentic cases as late as 1928.

The case that I am reporting is that of a negro child, aged four years. She was admitted to the hospital August 23, 1931, from the out-patient department, with a diagnosis of gonorrheal vaginitis. In June, 1931, she had undergone a vaginal operation elsewhere, for the removal of a large tumor. This tumor had not been submitted for pathologic examination.

Examination revealed a profuse, foul, brownish-yellow vaginal discharge which, according to her mother, had been present for some time, and since the operation had become much worse. The mucous membrane of the vulva was dark reddish grey. The inguinal lymph nodes were slightly enlarged. There was con-

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siderable urinary retention. On admittance to the hospital the patient's temperature was 100° F., pulse 130, and respiration 28.

Aside from the above mentioned facts nothing of note was found on physical

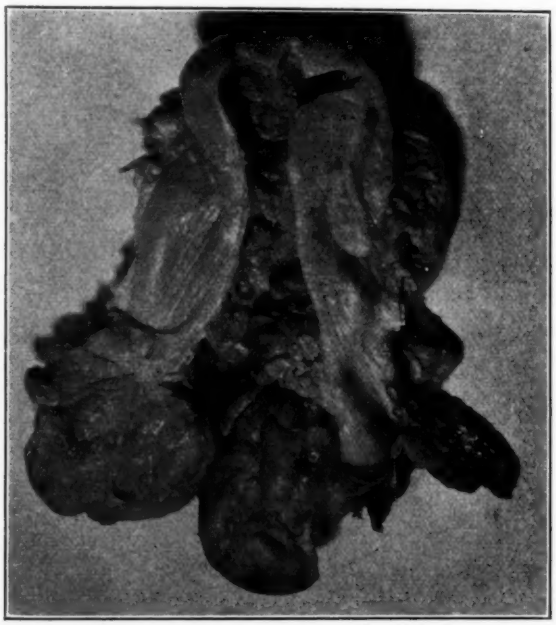

Fig. 2.—Large thin walled uterus; sacculated upper third of vagina and lower two-thirds packed with polypoid masses of the growth.

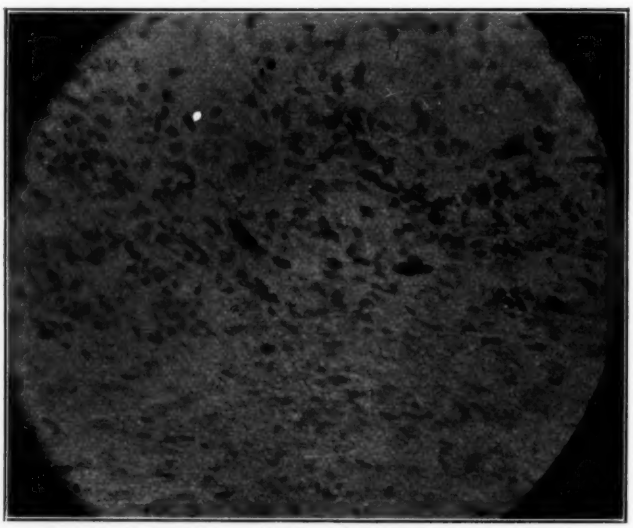

Fig. 3.—Section of the tumor showing cellular fibrous tissue and a few muscle fibers with large nuclei.

examination. Laboratory findings showed a leucocyte count of 31,000, with 84 per cent neutrophiles. Urine examination was negative except for a few pus cells. Smears from the urethral and vaginal discharges showed a mixed infection consist-

ing chiefly of colon bacilli. Later there was a decided increase of pus cells in the urine.

It was necessary to catheterize the patient during her entire stay in the hospital. Catheterization was difficult and painful and, as it became progressively more so, on September 7, she was anesthetized and a retention catheter inserted. At this time examination showed a large smooth tumor very much like a distended bladder, which reached to the level of the umbilicus.

The treatment consisted of warm soda bicarbonate douches and potassium permanganate, 1: 4000 dilution. The bladder was irrigated with boric acid. She was also given hot sitz baths and glucose and saline intravenously. She died September 11, 1931.

At postmortem examination there was found a marked pelvic peritonitis, hydronephrosis of the right kidney, and central necrosis of the liver. The uterus and bladder formed a large, lobulated, fluctuating mass that extended practically to the level of the umbilicus. The bladder was large and the mucous membrane was thick, polypoid, and very edematous. The urethra was considerably longer than normal, measuring 5 cm. The body of the uterus was the size of an adult's, and was filled with thick pea-green foul purulent liquid. It had a granular brownish-red necrotic lining. The uterine wall was thin, averaging 5 mm. in thickness. The vagina measured 13 by 10 cm., the upper 4 cm., forming a thin walled pocket similar to the uterus and in turn was filled with thick foul purulent liquid. The lower 9 cm. of the vagina was distended with a large soft edematous papillary tumor that was attached chiefly to the posterior vaginal wall, the anterior wall being entirely free. The tumors varied considerably in size (from 5 mm. to 5 cm.). Many of the larger ones showed superficial areas of necrosis and hemorrhage. Microsections showed the tumor to be composed chiefly of loose cellular fibrous tissue, scattered through which there were a few smooth and striated muscle fibers. The more superficial portions were diffusely infiltrated with inflammatory cells. Sections from the less edematous areas of the tumor showed a few large cells that had large oval, slightly elongated granular hyperchromatic nuclei.

CONCLUSION

A case of sarcoma botryoides with recurrence and death two and one-half months after surgical removal is described.

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PENETRABILITY OF TRANSPARENT MATERIALS FOR ULTRA-VIOLET RAYS*

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IN THE course of experiments with ultra-violet ray irradiation of tubercle bacilli, it became necessary to know which material used as cover for cultures would be most penetrable for ultra-violet rays, and thereby would permit the greatest bactericidal effect.

The following materials were tested by skin irradiation as well as culture irradiation experiments:

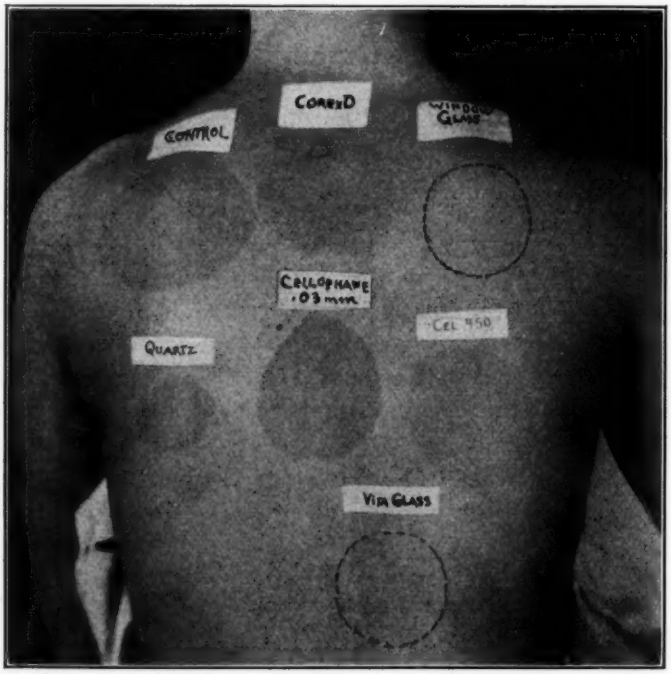

Fig. 1.

Cellophane	0.03 mm. thick (No. 450)
Cellophane	0.13 mm. thick (No. 1800)
Vitaglass	3.0 mm.
Window glass	3.5 mm.
Quartz glass	2.0 mm.
Corex D (Quartz glass)	2.0 mm.
Uncovered control	

It seemed to the authors that the results illustrated in Figs. 1 and 2 are quite indicative of the comparative value of these various transparent materials for culture experiments.

*From the Department of Medicine, University of Chicago.
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A mercury vapor lamp (Alpine Sunlight, Hanovia Company) was used for the irradiations.

Fig. 1, skin irradiation experiment. Irradiation time seven minutes; distance, 60 cm. The areas encircled by dotted lines are those irradiated through ordinary window glass and Vitaglass respectively. There was no erythema in either of these areas. The Corex D showed slightly more erythema than the cellophane 0.13 mm.

IRRADIATION TIME	CELLOPHANE .03mm (450)	CELLOPHANE .13mm (1800)	VITAGLASS	UNCOVERED CONTROL	WINDOW GLASS	QUARTZ GLASS	COREX D
10 min.	+ ± ±	## ## ##	## ## ## ##	+ + +	## ## ## ##	0 + +	+ ## ##
15 min.	+ 0 +		## ## ## ##	+ + +	## ## ## ##	+ + +	## ## ##

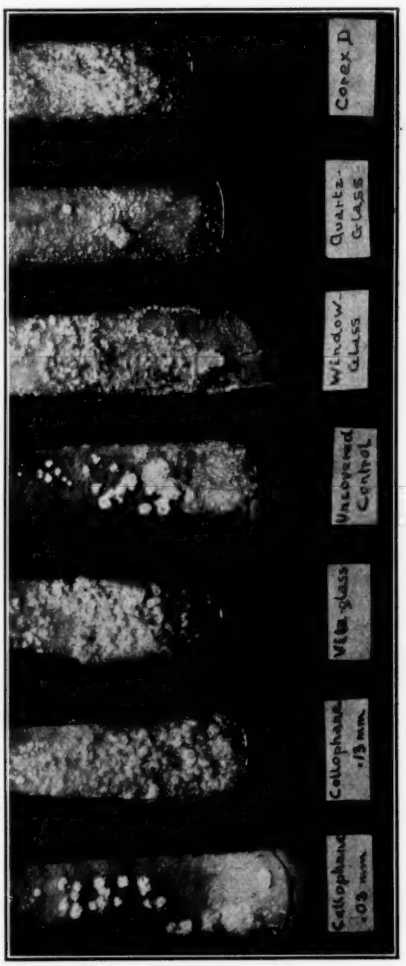

Fig. 2.

area, while the skin effect in the cellophane 0.03 area was equal to and even greater than that in the Quartz glass and uncovered control areas.

Fig. 2, culture irradiation experiments. Irradiation time fifteen minutes; distance, 30 cm. A suspension of tubercle bacilli of the virulent human strain H 119 was used. Eight milligrams of organisms were distributed in 1 c.c. of sodium phosphate solution M/15. The irradiation was carried out on 2 ½ mm. deep layers of the suspension in Stender dishes. Two parallel experiments with ten minute and

fifteen minute irradiation times respectively are recorded. (The fifteen minute period tubes of the experiment with the 0.13 mm. cellophane were lost and only the ten minute irradiation results recorded.) Three culture tubes were planted for each irradiation period; that is, a total of 6 tubes was available for each transparent medium. The additional five minutes hardly effected any difference in the growth of the organisms. The recorded readings of the cultures were made four weeks after their implantation. The tabulation in Fig. 2 records the growth in each culture tube: *0* indicating definite absence of colonies; ±, the presence of a few colonies; ++++, the growth of a maximum of colonies. Below each column in the figure, the photograph of a representative culture is shown. Only the window glass and Vitaglass cultures showed a maximum number of colonies in all tubes. With the 0.13 mm. cellophane this was nearly so, while Corex D produced a slight but definite inhibition of growth. With 0.03 mm. cellophane great inhibition was observed, in fact, as much as was present in the uncovered controls and in the cultures of bacilli irradiated through Quartz glass.

It will be noted that the findings by the culture experiments correspond well with those of the skin irradiation. Vitaglass, as well as window glass, stands out for failure to allow penetration of ultra-violet rays of erythema producing or bacteriocidal (tubercle bacilli) wave lengths, while thin cellophane of 0.03 mm. (or less) strength is recognized as an excellent material for ultra-violet ray transmission. It is hardly inferior to pure Quartz glass and of sufficiently low cost to allow its generous use in laboratory work. We have been using it in culture work for some time with results equally as gratifying as those reported by Johnson.* At the same time the experiment throws an interesting light on the value of the various transparent materials for general use in prophylactic and therapeutic irradiation.

BLOOD NONPROTEIN NITROGEN AND CREATININE IN NEPHRITIS AND PROSTATIC OBSTRUCTION†

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THE significance of increased concentration of creatinine in the blood has attracted considerable attention since it was first described in patients with uremia by Neubauer,[1] Folin and Denis,[2] and Myers and Fine.[3] Myers and Lough[4] stated that creatinine retention occurs, practically speaking, only in nephritis, and that values of 2.5 to 3 mg. per 100 c.c. are presumptive evidence of renal disease, 3.5 mg. indicating severe renal damage, values above 5 mg. indicating a fatal termination, usually within two months. These conclusions were reaffirmed by Chace and Myers,[5] Myers and Killian,[6] and Rabinowitch.[7] It was believed that the amount of increase of creatinine should be a more reliable index of the extent of renal functional damage than is that of urea or uric acid because of its endogenous origin and constant elimination under normal conditions. Myers, Fine and

*Johnson, F. H.: Cellophane Covers for Petri Dishes for Keeping Out Contaminations and Studying the Effects of Ultra-Violet Light, Science **73**: 680, 1931.

†From the Laboratory of Biochemistry and the Department of Medicine, Jefferson Hospital.

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Lough[8] observed that in early nephritis the blood uric acid was increased, the blood urea but slightly increased and the blood creatinine remained within normal limits, producing a staircase effect. This was interpreted as evidence that, in nephritis, elimination of uric acid is impaired first, that of urea later and that of creatinine last.

Subsequent investigations have in general failed to substantiate this interpretation of the mechanism underlying the relatively late rise in the concentration of creatinine in the blood in chronic nephritis. Watanabe[9] states that although creatinine appears to be excreted slightly more readily than urea under normal conditions, there would appear to be comparatively little practical difference in the ability of the kidney to eliminate these two waste products. This condition is supported by the recent work of Cope[10] who showed, by a comparison of blood urea and creatinine clearance studies, that in the nephritic subject the impairment of creatinine excretion approximately parallels that of urea excretion, the normal ratio between the two being roughly maintained for all degrees of renal damage. Berglund[11] likewise reported uniformity of retention of urea and creatinine in chronic nephritis. However, the serious prognostic significance of elevated blood creatinine values in chronic nephritis has been amply confirmed. This is not true of acute nephritis nor of acute exacerbations of chronic nephritis, obviously because of the possible transitory nature of the acute lesion in such cases. For example, Campbell[12] reported a case of acute bichloride nephrosis with recovery in which the blood creatinine had risen to 12.5 mg. per 100 c.c. Gatewood and Byfield,[13] reported a similar case with a blood creatinine concentration of 13.1 mg. per cent. Griffith and Cantarow[14] observed a patient with chronic glomerulonephritis in whom, during a period of acute exacerbation, the blood creatinine rose to 15 mg. per 100 c.c., subsequently falling to 1.4 mg.

The statement is frequently made that most of the extrarenal conditions which are associated with an increase in other nonprotein nitrogenous constituents of the blood have little or no effect on the creatinine concentration. Cases of pyloric or acute intestinal obstruction are, however, not infrequently encountered, in which rather marked grades of creatinine retention exist, as in two instances reported by Griffith and Cantarow.[14] From a practical standpoint, perhaps the most important group of extrarenal conditions associated with blood nitrogen retention is represented by obstructive lesions of the lower urinary tract, of which prostatic hypertrophy is the most common. Patch and Rabinowitch[15] reported 5000 observations of the relationship between the blood urea and creatinine concentrations in patients with renal disease alone, with obstructive extrarenal lesions alone, and with obstructive lesions complicated by organic disease. These observers found that whereas in nephritis there was a distinct parallelism between blood urea and creatinine values, in uncomplicated obstructive lesions the blood urea concentration was increased out of all proportion to that of creatinine. In those cases of urinary obstruction showing evidence of superimposed renal damage (casts) the findings were similar to those observed in nephritis. These observations are so different from our own experience that this study was undertaken for the purpose of comparing the relative degree of creatinine retention in nephritis with nitrogen retention and in prostatic obstruction with nitrogen retention.

PRESENT INVESTIGATION

The present series consists of 112 patients with nephritis (acute and chronic glomerulonephritis and nephrosclerosis) and 80 patients with urinary obstruction due to prostatic hypertrophy. Simultaneous blood total nonprotein nitrogen and creatinine determinations were carried out in each instance.

In the group of patients with nephritis, the blood nonprotein nitrogen concentration ranged from 40 to 289 mg. and the creatinine from 1.22 to 23.47 mg. per 100 c.c. In those with prostatic hypertrophy, the blood nonprotein nitrogen varied from 40 to 202.7 mg. and the creatinine from 1.4 to 15.82. The detailed findings are presented in Tables I and II. Tables III and IV, taken from the data of Patch and Rabinowitch,[15] are inserted for purposes of comparison. In comparing these data it must be realized that our figures represent total nonprotein nitrogen whereas those of Patch and Rabinowitch represent urea nitrogen. It may be noted that the relative degree of creatinine retention in our series of patients with nephritis is distinctly greater than was present in the cases reported by these observers. This discrepancy is difficult to explain. It may be that their cases of

TABLE I

NEPHRITIS

CASES	NONPROTEIN NITROGEN*	AVERAGE*	CREATININE*	AVERAGE*
24	40-50	45.82	1.22-2.34	1.87
15	50.1-60	54.0	1.52-3.26	2.07
9	60.1-70	65.36	1.22-9.44	3.71
5	70.1-80	73.60	2.06-4.02	2.63
7	80.1-90	87.39	1.78-8.20	5.82
2	90.1-100	95.25	2.74-2.76	2.75
9	100.1-110	103.77	3.28-8.10	5.64
8	110.1-120	116.67	3.22-10.90	6.99
6	120.1-130	122.80	3.42-10.10	7.45
12	130.1-170	146.8	3.34-18.00	10.50
4	170.1-200	187.39	11.52-17.40	14.02
11	200.1-289	252.21	9.64-23.47	15.07

*All values expressed in milligrams per 100 c.c.

TABLE II

PROSTATIC HYPERTROPHY

CASES	NONPROTEIN NITROGEN*	AVERAGE*	CREATININE*	AVERAGE*
23	40-50	43.72	1.40-2.58	1.96
14	50.1-60	55.48	1.88-3.06	2.34
9	60.1-70	64.89	1.97-6.00	3.23
7	70.1-80	75.11	2.06-5.16	3.15
3	80.1-90	84.52	3.44-5.34	4.54
4	90.1-100	94.50	2.36-4.50	3.47
2	100.1-110	105.86	4.50-11.50	8.00
4	110.1-120	115.92	6.90-10.58	8.68
3	120.1-130	125.58	7.10-9.44	8.21
6	130.1-170	144.68	4.86-15.82	9.83
4	170.1-200	184.11	8.35-14.30	11.19
1	202.70	202.70	11.76	11.76

*All values expressed in milligrams per 100 c.c.

TABLE III

BLOOD UREA NITROGEN AND CREATININE IN KIDNEY DISEASE
(AFTER PATCH AND RABINOWITCH)

UREA NITROGEN*	AVERAGE CREATININE*
20-50	1.2-1.89
51-90	2.21-2.93
91-120	3.10-3.71
121-170	4.28-5.86
171-200	5.86-7.87
201	9.30

*All values expressed in milligrams per 100 c.c.

TABLE IV

UROLOGIC CONDITIONS
(PATCH AND RABINOWITCH)

	UREA NITROGEN*	CREATININE*
Prostatism	168	2.4
Prostatism	230	2.43
Urethral Stricture	168	2.14
Carcinoma of Bladder	105	1.57

*All values expressed in milligrams per 100 c.c.

"kidney disease" included conditions other than nephritis. In our experience, creatinine values below 2 mg. per cent are not commonly observed in association with urea nitrogen values as high as 50 mg. in nephritis.

The difference is even more striking in the group of individuals with urinary obstruction due to prostatic hypertrophy. In this series the degree of creatinine retention was essentially the same as in the patients with nephritis. In fact, with total nonprotein nitrogen values below 130 mg. per 100 c.c., the blood creatinine was somewhat higher in the prostatic than in the nephritic group; in the former it rose on an average of 0.0513 mg. per cent for every milligram rise of nonprotein nitrogen above 40 mg., the corresponding rise in the latter group being 0.0452 mg. As the blood nonprotein nitrogen level rose above 130 mg. per 100 c.c., the creatinine values became more nearly equal, the degree of elevation in the prostatic group being 0.0695 mg. per cent for each milligram increase in nonprotein nitrogen, and in the nephritic, 0.0698 mg. per cent. No attempt was made in the present study to exclude from the prostatic group those individuals with superimposed renal lesions, a fact which might serve to explain these findings. However, the absence of serious renal damage in many cases with marked azotemia is evidenced by the fact that in 6 of the 11 cases of prostatic hypertrophy with nonprotein nitrogen values above 130 mg. and creatinine values ranging from 8.25 to 15.82 mg. per cent, both fell to within normal limits following relief of the obstruction to the outflow of urine.

DISCUSSION

In our experience purely obstructive urinary tract lesions cannot be differentiated from renal disease on the basis of the degree of elevation of blood creatinine.

In fact, in some instances, particularly in those cases with nonprotein nitrogen values below 130 mg. per 100 c.c., the rise in blood creatinine was slightly greater in the prostatic than in the nephritic group. A similar observation has previously been made in conditions such as acute intestinal obstruction, associated with prerenal deviation of water without demonstrable organic renal disease.

The discrepancy between our findings and those reported by Patch and Rabinowitch[15] is difficult to explain. These authors suggested that since the observed difference in creatinine values in nephritis and urinary obstruction could not be explained on the basis of different rates of excretion, the greater part of what is regarded as creatinine in nephritis is probably not creatinine but rather some unknown substance that gives the Jaffe reaction which, as is well known, is not specific for creatinine. In support of this belief they state that the observation of a positive diazo reaction in cases of advanced nephritis indicates the presence of some undetermined substance not present in the blood serum of individuals with urinary obstruction with a similar degree of urea retention. It is implied that this substance is perhaps responsible for the apparent difference in the degree of creatinine retention in the two groups of conditions. One must admit the doubtful identity of the substance or substances which are responsible for the reaction commonly attributed to creatinine in the blood. In fact, Behre and Benedict[16] go so far as to question the existence of creatinine in the blood. However, the data presented here do not support the hypothesis of the accumulation, in nephritis exclusively, of some specific substance giving the Jaffe reaction.

SUMMARY

1. Simultaneous determinations of nonprotein nitrogen and creatinine were made in the blood of 112 patients with nephritis and 80 patients with urinary obstruction due to prostatic hypertrophy in all of whom nitrogen retention was present.

2. It was found that, contrary to some previously reported observations, the degree of creatinine retention was practically the same in both groups of patients. With total nonprotein values below 130 mg. per 100 c.c. the average increase in blood creatinine was slightly greater in the obstructive than in the nephritic group.

3. It appears that no distinction can be made between purely obstructive urinary lesions and actual renal disease on the basis of the relative degree of blood creatinine elevation.

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STUDIES ON CALCIUM LEVULINATE WITH SPECIAL REFERENCE TO THE INFLUENCE ON EDEMA*

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IN SOME studies of calcium metabolism the question was raised not infrequently as to the effectiveness of various salts in elevating the calcium content of the blood.[1] Of interest were comparisons between calcium lactate and calcium gluconate in tuberculous patients. A satisfactory elevation (1 to 2.5 mg. per 100 c.c. of blood) above the "resting level" was noted following the administration of 3 or 5 gm. of calcium lactate on an empty stomach but this was accompanied usually by gastrointestinal manifestations (loss of appetite and nausea), especially if administration was continued (1 or 2 times daily) for three or four days. A smaller dose (0.5 to 2 gm.) had no constant influence on the blood calcium level even in prolonged administration (2 or 3 times daily), and in certain instances gastrointestinal symptoms occurred. In the use of the salt intravenously (5 or 10 c.c. of a 10 per cent solution) elevations from 1 to 2.5 mg. per 100 c.c. of blood (above the resting level) were noted. The injections were not entirely free from untoward effects even when the greatest care was used in administration. Of special concern was the frequency of local tissue reaction and vessel thrombosis. The subcutaneous and intramuscular injections were prohibited because of severe pain, inflammation and the danger of necrosis.

The variations in blood calcium following calcium gluconate administration (oral and intravenous) were similar to those in the use of calcium lactate except

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that relatively larger doses were required in order to obtain corresponding elevations. The untoward effects were less frequent and disturbing than in calcium lactate administration, except that diarrhea was not uncommon in prolonged use. In a few instances unexplained general reactions (chills and slight elevation of temperature) occurred during or after the intravenous injections. The subcutaneous and intramuscular injections were essentially free from untoward effects, but hypercalcemia was not striking except when more than 8 c.c. of a 10 per cent solution were used. Further experiences suggested criticisms. These were (1) low solubility in cold water (usually incomplete except in warm water), (2) the marked tendency to precipitation of 10 per cent solutions in ampoules, (3) the relatively low calcium content per gram of calcium compound (calcium gluconate contains 9.3 per cent Ca; calcium lactate 13.03 per cent Ca). The possibility that incomplete absorption (oral administration) was due in part to low solubility and that precipitation was responsible for the general reactions (intravenously) suggested that a study of other calcium salts would be of interest.

In a search of the literature it was found that Weintraub[2] attempted in 1894 to throw light on the relationship between levulinic acid and the occurrence of acetonuria. He used calcium and sodium levulinate experimentally and reported no toxic effects. In 1919 Frankel[3] considered that the salts of levulinic acid were toxic and referred to the studies of Weintraub as evidence, misinterpreting apparently this early work. Since the salt had certain theoretical possibilities, preparation was undertaken at the Chest Department of the Jefferson Hospital in 1930. In October of the same year a pure, neutral, soluble (30 per cent in cold water), stable (30 per cent in ampoules) compound containing 13 per cent of calcium was obtained.

PRELIMINARY OBSERVATIONS

Oral Administration.—Calcium levulinate was administered to animals as follows: Thirty female albino rats, each weighing 120 gm. were fed an average maintenance diet for fifteen days. The animals were apparently in good condition at the end of this period. A further series was selected for oral administration. The animals were caged individually and continued on routine care except that the food pans were removed each evening. In the morning a small amount of ground whole wheat with 0.65 gm. of calcium levulinate was fed to each animal. After consuming this ration the control diet with water was then fed ad libitum. None of the animals showed diarrhea, weakness, or loss of weight.

Subcutaneous Injections.—A sterile 10 per cent solution of calcium levulinate was prepared by intermittent sterilization. This was injected in varying doses into the abdominal wall. Four rats each received 1 c.c.; four 2 c.c. and four 3 c.c. every fifth day for four injections. There were no untoward effects and the solution was apparently well absorbed.

Intravenous Administration.—Injections of a 5 per cent sterile solution were made slowly into the coccygeal veins of seven rats. These injections were made on alternate days until five doses were administered. The doses varied from 1 c.c. to 3 c.c.; in one animal the dose was 5 c.c. No harmful effects were noted except following the administration of 5 c.c. when the animal exhibited signs of collapse. Recovery occurred rapidly and subsequently four doses of 3 c.c. were injected without causing reaction.

FURTHER OBSERVATIONS

The compound was administered to far advanced tuberculous patients (service of Dr. N. Blumberg, Philadelphia General Hospital) orally (1 to 2 gm. dissolved in 50 c.c. of water once or twice daily) and subcutaneously (3 to 5 c.c. of a 10 per cent solution two times weekly for four doses). The longest period of oral administration was three weeks, the average being eleven days. Three patients experienced transient loss of appetite and nausea on the third day and administration was discontinued. The subcutaneous injections (seven patients) were followed by pain and discomfort at the site of administration, which lasted from a few hours up to three days, but there was no evidence of necrosis.

Studies to determine the immediate effects of calcium levulinate were undertaken in a series of 14 tuberculous patients at the Jefferson Hospital. The dose varied between 5 and 10 c.c. of a 14 per cent solution (three times weekly). Four patients each received 7 injections and the rest four injections. Clinical data on local and general reactions and variations in pulse rate, respiration and temperature were obtained before and at intervals for several hours after the injections. These showed no characteristic change except transient reduction in the respiratory rate, dryness of the throat and a sensation of warmth throughout the body during and immediately following administration. These manifestations were similar to those noted in the use of parathyroid hormone.[1] Slight soreness and inflammatory reactions lasting for two or three days appeared at the site of injection in five patients. These were due to extravasation of the salt into the tissues. Blood calcium determinations made at intervals following oral and subcutaneous administration showed no striking or characteristic change. However a sharp elevation of blood calcium (1.5 to 4 mg. per 100 c.c. of blood) above the "resting level" usually followed the intravenous injections. The "peak" occurred from three to ten minutes after administration and a gradual return to normal occurred within three hours. Definite reduction (one to three minutes) in the clotting time of the blood was noted in six patients receiving the salt intravenously. The maximum effect was noted within thirty minutes after administration.

An interesting clinical effect occurred in three patients with marked edema of the legs and abdominal wall. In the first case more than 500 c.c. of fluid exuded from a bed sore within two hours following injection of 8 c.c. of a 14 per cent solution and subsequent accumulations responded in a similar manner. In the second case with urinary output of less than 250 c.c. daily there was marked diuresis and reduction of edema within a few hours after administration. The condition returned in these patients and similar effects were noted after further injections. The third case with marked displacement of the heart due to pulmonary fibrosis and with swelling of the legs, abdomen and hands, experienced no effect on edema following a period of digitalization. He received 2 injections of calcium levulinate daily for three days and striking reduction of swelling occurred.

Since the immediate effects of calcium levulinate were nontoxic, oral, intravenous and intramuscular administration* was undertaken in 26 patients with far advanced pulmonary tuberculosis to determine the effects of prolonged administration. Possible influences on tuberculous processes were not studied. The powder

*The calcium levulinate (cavulin) was manufactured by Smith, Kline and French Laboratories.

for use orally was flavored with lime and mixed with lactose. The latter was employed according to the studies of Bergeim[5] who found that absorption of calcium carbonate was increased in an acid medium. The solutions for the injections contained from 3 to 14 per cent calcium levulinate and the dosage varied from 1 to 10 c.c. The oral administrations (2 gm. daily) were continued in 8 patients for six weeks. The intramuscular injections (2 injections weekly) were given to 7 patients for three weeks and the intravenous injections (1 to 4 weekly) to 11 patients up to twelve weeks. The greatest number of intravenous injections was 43 in one patient sixty-three years of age. The immediate symptomatic effects were the same as previously noted, except that patients receiving prolonged intravenous administrations experienced transiently, an increase of strength and decreased expectoration. These lasted usually for a few hours after each injection.

Blood calcium determinations were made at intervals during treatment. So far as determined there was no evidence suggesting that the prolonged oral and subcutaneous administrations maintained a constant elevation of the blood calcium of more than 1 mg. per 100 c.c. of blood. In the majority of instances no definite change was noted. In the intravenous administrations transient elevations followed the injections.

It appears from the present investigation that calcium levulinate is essentially nontoxic and may be considered for trial in conditions in which calcium therapy is indicated. Certain features suggest definite advantages over other calcium salts. Of special interest is the high degree of solubility and stability in solution as compared with calcium gluconate and the relative freedom from severe local reactions which are not uncommon when extravasation occurs in calcium lactate administration. As in previous experiences with calcium salts administered orally to tuberculous patients in toleration doses, the effect of calcium levulinate on the resting blood calcium level was not striking. Incidentally this raises questions as to the rate and degree of absorption and mobilization of calcium following various methods of administration. These are pertinent in considering the large reservoir of blood and tissue fluids which receive a comparatively small dosage and because excretion under certain circumstances may almost parallel absorption. It would appear in general that intravenous administration should be employed when a sharp elevation of blood calcium is desired. In cases with mild calcium deficiency the simple administration of foods rich in calcium values (800 c.c. of milk contain more calcium than 13 gm. of calcium gluconate) and the use of sunlight, cod liver oil, and viosterol should be effective. Mobilization may be increased by injections of parathyroid hormone.

Since this investigation was undertaken, Bennett[5] reported the use subcutaneously of a 3 per cent solution of calcium levulinate and calcium gluconate in tetany. The substance was rapidly absorbed without untoward effect and arrest of the tetany was secured. Geville and Dodds[6] used calcium levulinate intravenously (20 c.c. of a 10 per cent solution) in jaundiced patients without untoward effect.

CONCLUSIONS

1. Calcium levulinate contains practically the same amount of calcium as calcium lactate (about 40 per cent more than calcium gluconate), is soluble and stable in 30 per cent solutions.

2. In animal and clinical investigations (tuberculous patients), calcium levulinate was nontoxic and effective intravenously in increasing the calcium content of the blood above the normal level. A reduction in the clotting time of the blood occurred in a certain instance.

3. The immediate and striking influence on edema in three patients is noted. Symptomatic manifestations such as dryness in the throat, sensation of warmth, and decreased expectoration suggested "calcium effect."

We are indebted to Dr. W. L. Haight and Mr. R. J. Titherington for valuable assistance in this study.

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LABORATORY METHODS

DEHYDRATED EGG MEDIA FOR ANAEROBIC CULTIVATION AND DIFFERENTIATION*

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EGG media have long been utilized for the cultivation of bacteria, and a variety of formulas have been developed according to the peculiar individual requirements, such as the Dorsett, Petroff, Goldberger and Robertson media.

Kahn[1] has utilized coagulated egg albumen cubes and a modification of the Robertson alkaline egg in the identification of a group of single-cell strains of anaerobes. We have followed these procedures, but due to the time required for preparation, and loss from blowing of plugs, a study was made to develop a satisfactory dehydrated substitute.

Through the cooperation of the Difco Laboratories three forms of dehydrated preparations were made available; respectively, whole egg, egg yolk, and egg albumen, each ground to a fine dry powder. The latter two were carefully separated to represent only the yolk or the albumen.

Each of these may be prepared by placing approximately 0.5 gm. in a dry tube, preferably 8 by 5/8 inches, to which is added 10 mils. of distilled water or nutrient broth. A slight stirring brings the powder into suspension, after which the tubes may be autoclaved by the usual process without tendency to blow, or even to wet the plugs. The medium thus prepared has a reaction very close to P_H 7.2, which Kahn and others regard most satisfactory, and presents a loosely flocculent column of solids some 3 cm. deep with about 2 cm. of fluid above it.

These tubes may then be inoculated deeply by loop, or preferably by pipette, with anaerobes from deep brain stock cultures, or from stock cultures in the whole egg medium which serves as an excellent medium for maintaining cultures over long periods. No vaseline or other seal is required, which is a great advantage in anaerobic cultivation, although, if desired to determine the amount of gas formed, the "vas-par" seal of Hall may be utilized.

The entire procedure is less tedious and more cleanly than that involved in the preparation of deep brain and similar media, although it is not to be inferred that this disparages the many valuable features of the latter. The fact that this egg medium may receive routine autoclaving, with resultant insured sterility, and no loss from blowing, considerably reduces labor and wastage.

In these egg media the various anaerobes produce characteristic reactions, only a few of which will be outlined below. These reactions consist of varying degrees of proteolysis, color changes, consistency of medium and gas formation.

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[1] Kahn, M. C.: Pub. Cornell Univ. Med. Coll. **17**: 155, 1919-1922.

In addition to these reactions, the use of reduced iron (J. T. Baker Iron Powder Reduced by Hydrogen), as suggested by Hastings and McCoy at the Baltimore meeting of the Society of American Bacteriologists, both facilitates anaerobic growth and intensively accentuates blackening by the proteolytic organisms. For this purpose as little as 0.125 per cent sharply blackens with such organisms as Cl. sporogenes and Cl. putrificum. Larger amounts, up to 0.5 per cent seem to increase the growth and, of course, also the degree of blackening. Tubes inoculated with nonproteolytic organisms fail to show blackening, and instead become reddish-brown, particularly near the surface, presumably with the formation of ferric oxide.

Lead acetate may also be incorporated to detect H_2S. Its toxicity, however, limits its use to a maximum of 0.2 per cent concentration, while 0.05 per cent produces an intense blackening with Cl. sporogenes, Cl. tyrosinogenes, and others.

As noted above, either distilled water or broth may be used as the diluent. With the latter (Difco Tryptone Broth) the physical state is more flocculent, and the growth rate is considerably accentuated, due to the accessory food substances in the medium. Thus, Cl. tetani in egg albumen with broth shows gas and bacterial turbidity within twenty-four hours, while with distilled water only the slightest evidence of growth appears within ten days.

Since the purpose of this report is primarily to describe the medium and its generally useful features, the reactions of only three representative organisms will be included:

1. *Cl. sporogenes, an Actively Proteolytic Type.*—Whole egg in tryptone broth, active growth at twenty-four hours; decomposition evident, with 1 cm. of gas under "vas-par" seal; bright yellow initial color changing to a dirty gray-yellow. Prolonged incubation gives about 2.5 cm. of gas, and leaves about 0.2 cm. of grayish amorphous deposit after forty days. Odor is extremely foul. Sporulation is abundant at forty-eight hours. If 0.5 per cent reduced iron or 0.05 per cent lead acetate is incorporated the fluid becomes very black, with a black sediment.

Whole egg in distilled water, changes as noted above, but much less active. Blackening of iron is approximately the same.

Egg yolk, reactions scarcely distinguishable from those in the whole egg. Growth and gas formation seem even to be accelerated, and the amount of residual sediment at forty days is almost nil.

Egg albumen in tryptone broth, active growth at twenty-four hours, with evident decomposition as shown by slight translucence of the solids. About 0.5 cm. of gas at twenty-four hours, with a maximum of about 1.5 cm. Albumen completely decomposed at ten days, leaving only a slight grayish deposit. If iron or lead acetate are added blackening is evident at twenty-four hours, and increases to a maximum at three days.

Egg albumen in distilled water, no evidence of growth during the first twenty-four hours, but marked at forty-eight hours, and then generally following the character of the reactions in broth.

Cl. putrificum, Cl. botulinum and Cl. tyrosinogenes fall in this class, though in general much less active. Cl. tyrosinogenes produces an abundance of tyrosin crystals in all forms of the egg, but much more abundantly when tryptone broth is used as the diluent.

2. *Cl. tetani, a Feebly Proteolytic Type.*—Whole egg in tryptone broth, growth and gas delayed until forty-eight hours; bright yellow changes to dirty grayish-yellow at about ten days. Under "vas-par" seal forms a maximum of about 1 cm. of gas. Further reaction consists solely of a fractional separation of an upper layer of dirty gray flocculent material over a lower mass of yellow yolk. The odor in this medium is only moderately fetid. The initial mass of solids is reduced by only about ¼ its volume in forty days. Sporulation is abundant at five days.

Whole egg in distilled water, growth greatly delayed and very inactive. Evidently egg alone is not well suited to the nutritional requirements.

Egg yolk in tryptone broth, as above, with practically no change in character or volume.

Egg yolk in distilled water, growth greatly delayed, with practically no change other than slight graying. Frequently transfers fail to show any evidence of growth.

Egg albumen in tryptone broth, no evidence of growth usually until fourth or fifth day, when gas appears. Very slow proteolysis occurs, and in presence of 0.5 per cent iron, beginning at about ten days, a mottled blackening appears in the whitish medium, increasing to maximum at about thirty days. Proteolysis is only partial at forty days.

Egg albumen in distilled water, usually no growth nor perceptible change in medium. Evidently the organism cannot successfully initiate growth in solely an albumenous medium.

Cl. edematiens and Cl. edematis-maligni have these same general cultural reactions.

3. *Cl. welchii, a Nonproteolytic Type.*—In all forms of egg, with broth or distilled water, produce active growth with abundant gas in twenty-four hours. The solids are raised to the top with the emeshed gas bubbles. No change in color or consistency occurs. No odor is observed. Sporulation is very slow, in fact of 4 strains tested spores have been observed in only 2 tested up to ten days. Microscopically evidence of sporulation is uncommon within thirty days.

All forms of the egg medium remain unchanged in color, even when iron or lead acetate are included.

No other anaerobe which we have studied shows such reactions.

SUMMARY

A series of three forms of dehydrated, powdered egg preparations are described. When prepared in tryptone broth, the whole egg presents an easily prepared medium very suitable for maintenance of stock cultures of the common sporulating anaerobes.

All forms, especially when combined with reduced iron in suitable concentration, show characteristic proteolytic changes which suggest useful application to the identification of species.

Characteristic reactions of three representative species are described.

A ROUTINE CLINICAL EXAMINATION FOR TUBERCLE BACILLI IN MICROSCOPIC NEGATIVE SPUTUMS BY VARIOUS CULTURE METHODS*

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THE tubercle bacillus grown in the incubator requires certain fundamental conditions approaching that of body environment. These must be maintained over a considerable period of time in order that small numbers of bacilli may multiply sufficiently to be discerned by the usual means of microscopic or macroscopic detection. In an earlier report[1] it was shown that to readily detect tubercle bacilli in smears of sputum and in sections, it required at least about 100,000 bacilli per cubic centimeter. It was also shown that the guinea pig[2] was a reliable delicate test for small numbers (from 10 to 100) of human and bovine tubercle bacilli and that culture methods could hardly be expected to attain more than equal efficiency. This, however, was achieved by preliminary treatment with an equal volume of 6 per cent sulphuric acid or better, a 5 per cent oxalic acid solution[3] to destroy undesirable contaminators and then planting the washed residue on a satisfactory sterile nutrient medium, the crystal violet potato cylinder medium. The conditions of the medium including its moisture, neutrality, isotonicity, sterility, and general physical composition all play a part in maintaining conditions suitable for the proliferation of small numbers of bacilli. In earlier studies[4] it was shown that a medium (such as glycerol agar or Long's synthetic medium) might be well suited for supporting the growth of heavy plantings of tubercle bacilli, such as are obtained from sputums positive on microscopic examination, and that they might even appear to grow on these mediums more luxuriantly and more rapidly in heavy plantings than on good nutrient mediums. Poor nutrient mediums made of soup stocks or simple chemical salts, or good nutrients vitiated by dilution with inert substances like agar, were entirely unsuited for supporting the growth of small numbers of bacilli such as are found in negative specimens of smears or sections, using microscopic examination as the criterion.

As a result of earlier studies on the isolation of tubercle bacilli for diagnostic purposes a sulphuric acid or oxalic acid crystal-violet glycerol water potato culture method was described. The procedure consisted briefly, of destroying the contaminators with an equal volume of 6 per cent sulphuric acid or 5 per cent oxalic acid, mixing well with the suspected specimen and incubating for thirty minutes at 37° C. The material was then diluted with ten volumes of sterile 0.9 per cent saline solution to remove the acid and the residue after centrifuging was planted on the neutral potato medium. Since these experiments were performed and the method recommended, it has been found that the saline washing does not suffice to remove the acid from the tissue residue and that the material when planted may still register a low P_H (2-4), an acidity which may possibly persist in the planted

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specimen for some time after incubation is begun.[5] It was also found that a P_H below 4 would destroy the bacilli within a few days at incubator temperature. For this reason it appeared desirable to study the effect of chemical neutralization as opposed to washing alone for suspected sputum specimens.

It is obvious that only empiric material, "suspected sputums," was available for such a study since seeded control sputums could hardly be used without introducing the factor of bacilli already present in the sputums. It is our experience also that results obtained with studies pursued with sputums positive in microscopic smears are of no value in studying methods for isolation, or for studying the value of nutrients, for small numbers of bacilli. The striking results from the study with sputums negative by the microscopic smear test, made this report appear highly advisable even though the original intention of the experiments did not include such plans.

We have now completed studies (to be published shortly) with nutrient mediums for small plantings of tubercle bacilli and have compared these with the sterile potato cylinder originally recommended. It has been found that simple inspissated glycerol (3 per cent) water (25 per cent) egg yolk medium without the addition of broth or other diluents compares favorably with the potato medium. It was for this reason that the potato medium and egg yolk medium were used in these studies. In order to further check the mechanical manipulations, preparation of media, and performance of tests, all specimens were tested by two independent and separate groups of trained workers who also used separate incubators. One hundred suspected sputums negative by microscopic examination were used for the tests. These were obtained from cases in which routine monthly smear examinations had been made. Of these, 32 per cent had never been positive by smear during residence in the sanatorium, 5 per cent had not been positive for over two years, 11 per cent had not been positive for over one year, and 52 per cent had been negative from four to twelve months prior to culture test.

The specimens tested by culture were carefully examined in smear to determine that they were negative for the purpose before proceeding to use them for this experiment. The method used for destroying contaminators consisted briefly of mixing 1 c.c. of a well homogenized sputum (homogenized by means of an electric vibrator beater) with 1.5 c.c. of 5 per cent pure oxalic acid solution. After shaking well, the mixture was incubated (at 37° C.) for about forty-five minutes (previous tests having shown that a time leeway within sixty minutes is permissible). One sample was then washed with ten volumes of sterile 0.9 per cent sodium chloride solution and centrifuged, while another was neutralized to P_H 7.0, using brom thymol blue as indicator, and centrifuged. The sediment, after decanting of the supernatant liquid, was again mixed with 0.9 per cent saline solution and planted on a number of tubes of potato and egg yolk medium. Sodium hydroxide as a reagent to destroy contaminators, was tested in various ways with the same specimens of sputum. It was used in equal volume and 1.5 volumes, of 3 per cent and was left unneutralized (only washed), and in comparable tests was neutralized with sulphuric acid solution.

The pertinent results of this study are included in Tables I and II.

An examination of Table I reveals that neutralization is not necessary when dealing with sputum. However, tests with tissues and blood so alter the conditions

TABLE I

A COMPARISON OF THE WEEKLY POSITIVE RESULTS OBTAINED BY TWO SEPARATE GROUPS OF WORKERS WITH ONE HUNDRED MICROSCOPIC NEGATIVE SPUTUMS USING OXALIC ACID AND POTATO OR EGG MEDIUM AS TEST METHOD

METHOD USED	NEW POSITIVE FINDINGS OBTAINED AT WEEKLY READINGS						TOTAL POSITIVES
	2 WK.	3 WK.	4 WK.	6 WK.	9 WK.	12 WK.	
Oxalic acid—potato medium	1*	5	25	9	7	3	50
	0	9	25	12	4	1	51
Oxalic acid—neutral with $NaHCO_3$—potato medium	1	11	14	14	9	1	50
	0	8	33	8	4	1	54
Oxalic acid—egg yolk medium	4	18	13	8	3	1	47
	2	21	7	11	2	3	46
Oxalic acid—neutral with $NaHCO_3$—egg yolk medium	3	23	13	3	9	6	57
	1	23	14	7	2	1	48

*The upper figure throughout the table records the positive findings by one group of workers while the lower figure records those of the second group.

that with these materials, neutralization is necessary. The data on this will be published shortly. The character and amount of residue from acid treated sputums make early reaction adjustment from the neutral nutrient medium possible, which is not the case with tissues and blood.

It is also to be noted that the two groups of workers obtained approximately the same percentage results in spite of the lack of uniformity of empiric specimens due to the nature of the tuberculous material excreted in the sputum. It is also to

TABLE II

THE SIGNIFICANCE OF CONTAMINATIONS IN TESTING ONE HUNDRED NEGATIVE MICROSCOPIC SPUTUMS FOR TUBERCLE BACILLI

METHOD USED	NUMBER OF TUBES CONTAMINATED*	COMPLETELY CONTAMINATED OF A TOTAL OF 100 SPUTUMS	TUBES POSITIVE FOR TUBERCLE BACILLI*
Oxalic acid—potato medium	14**	0	125
	8	0	136
Oxalic acid—neutral with $NaHCO_3$—potato medium	12	1	128
	12	1	135
Oxalic acid—egg yolk medium	24	3	109
	24	0	119
Oxalic acid—neutral with $NaHCO_3$—egg yolk medium	30	2	118
	14	1	129

*Each group used 300 tubes of culture medium for the 100 sputums and for each method. The total tubes of culture medium used by both groups for all the tests were 2400.

**The upper figure given in the tabulations represents the findings of one group of workers while the lower figure represents the findings of the second group of workers.

be noted that the potato cylinder medium and the inspissated egg yolk medium both proved to be good nutrients, and about equal in value, by quantitative tests with fine suspensions of tubercle bacilli, yielded about the same percentage positive findings with the negative microscopic sputums.

Since the elimination of contaminations is an important phase of the procedure of isolating pure cultures of tubercle bacilli for diagnostic purposes the data presented in Table II are particularly pertinent in revealing the value of oxalic acid solution for this purpose.

The agreement noted between the two groups of workers both in contaminations and positive findings for tubercle bacilli was quite striking in view of the empiric nature of the material tested. If figured in percentage of the total tubes contaminated it was for the first group 7.2 per cent, and for the other 4.8 per cent; while the positive cultures for tubercle bacilli obtained by the first group was 39.9 per cent of the tubes of medium used, and by the second group was 43.2 per cent. It is interesting also that only six sputums of the 100 sputums examined by four methods by the first group contaminated totally by one of the methods, and that for the second group only two sputums contaminated by one of the methods. In no case was the individual sputum contaminated in all four methods.

The empiric nature of the tested material, the sputums, is attested by the results in contaminations as well as in positive cultures obtained, bearing out the well known physical nature of tuberculous material. This is further borne out by the fact that although the percentage positive findings by each group was about equal, this equality attained as a result of the fact that five sputums found negative by the first group were positive for the second, and another five sputums were positive for the first group and negative for the second. In many cases (15 per cent of the sputums) where twelve tubes were planted using four methods, only one tube revealed a positive culture. If the positive results of the two groups of workers using the four methods were taken in total 71 per cent of the 100 sputums were positive by culture.

In the case of apparently negative findings we have occasionally found that a careful scrutiny of the surface of the media would yield a positive solitary macroscopic colony, which easily passes unobserved in poor light or with hasty superficial examination. This was particularly so for the potato medium but also was true with the egg yolk medium. Trained workers rarely failed to detect such colonies. In verification of earlier observations[2] the tests with 3 per cent sodium hydroxide to destroy contaminators proved this reagent to be far less efficient than the oxalic acid. Because of the contaminations, in addition to the greater toxicity of the sodium hydroxide for the tubercle bacilli, the percentage positive findings for tubercle bacilli were considerably less than the tests with oxalic acid. The contaminations in this series of sputums treated with sodium hydroxide neutralized with sulphuric acid ran from 16 per cent on the potato medium to as high as 52 per cent on the egg yolk medium. For this reason a tabulation of the results with the sodium hydroxide method was omitted.

DISCUSSION AND CONCLUSIONS

In practical clinical medicine a positive diagnostic finding is in the majority of cases of far greater significance than a negative observation. It was previously

pointed out that the finding of tubercle bacilli in pathologic materials might be considered in the light of a certified diagnosis in tuberculosis[1] provided the error of calling all acid-fast bacilli, tubercle bacilli be guarded against. Specimens from certain regions of the body yielding acid-fast bacilli such as is exemplified in sputum usually prove reliable in being designated as tubercle bacilli. Therefore, the microscopic findings in stained smears from certain regions assume practical importance when positive. The fact, however, that it requires the presence of large numbers of bacilli (at least 100,000 per c.c.) in a specimen before they can be readily discerned in the stained smear makes negative findings by this method obviously extremely unsatisfactory from a clinical or public health standpoint, and makes the so-called negative specimen assume importance. The highly susceptible guinea pig and delicate culture method both have proved valuable for discerning tubercle bacilli as contrasted to acid-fast bacilli and especially for discerning the presence of small numbers (as few as 10 to 100). The culture method possesses the advantage of ready accessibility and economy of performance, but both the guinea pig and culture method have the disadvantage of requiring time for obtaining results. Yet the time element in a chronic disease does not assume the proportions it would in an acute disease and cannot counteract the importance of reliable positive findings. The importance of applying the more sensitive culture test as compared to the microscopic smear test is evident when it is realized that 71 per cent, in this series, of sputums which had been negative by smear for intervals of from four months to over two years proved positive on a single examination by culture. There is no doubt that a repetition on another specimen from the 29 cases negative on single test would probably reveal additional of these as positive, in view of the fundamental nature of tuberculous material.

SUMMARY

Of 100 sputums, negative, for from four months to over two years on monthly reexamination by the microscopic smear test, 50 per cent were found positive by a single culture test. When several delicate culture methods (potato medium and egg yolk medium) were used and the studies were carried out by two groups of workers, 71 per cent positives were obtained. The finding of positives by one group and not by another is understandable on the basis of the lack of uniformity of empiric specimens, due to the nature of the tuberculous material. This makes accurate scientific observations with routine sputa of little value for comparison of methods or media unless verified by accurate quantitative studies. It does not, however, vitiate the practical importance of the positive findings in a high percentage of clinical specimens found repeatedly negative by smear, and suggests a more extended use of culture methods in diagnostic and public health work in spite of the apparent disadvantage of time delay. The importance of repeating a test, when negative, is obvious and requires no comment.

The technical details of this study were conducted with the assistance of C. D. Vidal, L. D. Miller, Ray Stoner and Martha Brown.

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UNBREAKABLE TUBES FOR SEROLOGIC WORK AND FOR THE SHIPMENT OF BLOOD SPECIMENS*

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TO ELIMINATE breakage in transit and in the laboratory, I have devised a light-weight, inexpensive, and unbreakable tube for the shipment of blood specimens.

The tube is made of nonexplosive, though combustible celluloid. It measures 75 by 15 mm. and holds 10 c.c. of the blood. A properly softened cork stopper, quality XX, size 5, short, fits into this tube firmly enough to prevent any leakage in

Fig. 1.—Unbreakable tube and mailing carton.

transit regardless of rough handling. I have tested the dependability of the tube and the security of the cork stopper by throwing the loaded mailing container vigorously against the ceiling of the laboratory and allowing it to drop to the floor, and by throwing it from one end of the room to the other with force. The tube was found unharmed, and the stopper securely in place. No leakage was observed.

The blood can be kept in the washed and sterilized tubes from one to three days without bringing about any change in the original immunologic properties of the specimens. Tubes 75 by 10 mm. made of the same material were used in carrying out complement fixation (cold over-night incubation) tests, and tubes 75 by 7 mm. were used in the precipitation tests. The results could be read with the same ease as in the glass tubes. No hemolytic or anticomplementary reactions were experienced in the case of complement fixation tests and no pseudo-precipitations were observed.

The tubes are cheaper than the glass tubes, if purchased in large quantities, and since they are not fragile they last indefinitely, and afford a worth-while economy.

When first obtained, the tubes are soaked in warm soapy water, gently swab-

*From the Clinical Laboratory, Public Health Institute.
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bed with a soft hair brush and thoroughly rinsed inside and out in a continuous stream of tap water. The tubes are then placed mouthside down into galvanized wire baskets and drained to dryness. The baskets are then placed inside a specially constructed sterilizing chamber. On the bottom of this sterilizer is a shallow enamel tray, into which is placed a folded cotton towel or sheeting. This is soaked with full strength formaldehyde, and the door of the sterilizer is tightly closed. The tubes are left under the influence of the vaporized formaldehyde for twelve to twenty-four hours. This period was found to be ample for the complete sterilization of the tubes. A sufficient number of cork stoppers, thoroughly washed, contained in a cotton gauze sack are sterilized at the same time. At the end of twelve to twenty-four hours the tubes and the stoppers are removed from the sterilizing chamber and the tubes are carefully stoppered.

Each tube is equipped with an identification blank. It is properly packed in a container lined with a piece of corrugated paper. This is placed in a carton, and is ready for shipment. Used tubes are cleaned and sterilized as described above.

It must be remembered that these tubes, though not explosive, are combustible. They must not be dried inside or over the dry-air gas oven, nor must they be exposed to the open flame. The tubes can be sterilized as many times as may be required by the formaldehyde-vapor method, but will not stand repeated sterilization by steam or by boiling.

Fig. 1 shows the complete outfit necessary for mailing blood specimens.

A METHOD OF COLLECTING AND PRESERVING SMALL BLOOD SAMPLES FOR GLUCOSE DETERMINATIONS*

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THE present study was undertaken in connection with the glucose tolerance tests of insurance companies. A method was desired, the accuracy of which would not depend on a definite measurement of the sample at the time of collection. In the procedure offered the technic for collecting is simple and may be undertaken by an untrained person. For this reason it may have applications other than its present one. A diabetic patient, for example, should find it as easy to take a finger-tip sample of his blood as to give himself an insulin injection. To diabetic specialists it is suggested as an easy way to keep in touch with patients living at a distance. It may also be found a serviceable device to have in a hospital laboratory for collecting and transferring samples.

A 0.1 c.c. capillary pipette lined with preservative salts is used for the collection, the blood being sealed inside the pipette by means of a rubber band stretched over the ends. For this purpose the usual Folin micro-blood pipette[1] is shortened by cutting off the blunt end, and an extra graduation is added about 3 cm. beyond the 0.1 c.c. mark. The blood sample is collected to this second graduation and the measurement is left to the analyst at the time of testing. The procedure is as follows: Make the lancet puncture in the finger-tip, allow the blood to flow into the

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pipette, tip it back and forth a few times to dissolve the preservative lining and attach the rubber band. At the time of making the analysis measure the sample by discarding the excess blood and analyze it in the same way as a fresh sample.

The pipettes selected were 16 cm. long and it was found that the corresponding rubber bands must be 1 cm. in width and 9.5 cm. in length. If longer or narrower the bands did not fit the pipettes snugly and permitted some evaporation of the blood column inside. If shorter they were too tight and might be punctured by the tips of the pipettes.

As a preservative Lax and Szirmai[4] recommended the use of sodium fluoride and mercuric chloride. According to their report these two salts, in the proportion of sodium fluoride, 1 per cent, and mercuric chloride, 0.1 per cent, prevented glycolysis in blood samples for periods up to a month and had no reducing effect on the testing reagents.

Our investigation was complicated somewhat by the difficulty of getting a measured amount of the salts into the pipette. Aqueous solutions were found impracticable because the drying process was so slow at room temperature, and if oven drying was resorted to the solution expanded and a large proportion overflowed from the openings of the pipette. For this reason it was decided to try a suspension instead of a solution and the sodium fluoride was precipitated from a concentrated aqueous solution by the addition of 95 per cent alcohol. The suspension could then be sucked into the pipette to the second graduation, since this was to be the measure of blood collected, and with the aid of a towel applied at the tip most of the alcohol-water mixture could be drawn off, leaving the deposit of salt inside. This could then be quickly distributed and dried by passing a gentle current of air along the bore of the pipette.

The mercuric chloride, however, being soluble in alcohol, could not be so precipitated. When the two salts were dissolved together in water, and alcohol was added, some of the mercuric chloride was brought down, but in a reduced state, and on standing further reduction with consequent darkening of the precipitate was noted. Blood samples treated with this precipitate showed a loss in glucose value indicating that the mercuric chloride had not been available.

It was then decided to adopt the procedure outlined above for the fluoride salt but to add the mercury in alcohol solution to the previously precipitated fluoride suspension. And because most of this alcohol solution would be poured out of the pipette after its introduction, the concentration of the mercury was increased from 0.1 per cent to 1 per cent. Enough then remained in the pipette after decanting to approximate the desired amount.

PREPARATION OF THE SUSPENSION-SOLUTION

Dissolve 0.5 gm. of sodium fluoride in 15 c.c. of water and rinse into a 50 c.c. volumetric flask, using as little water as possible for the rinsing. Add 95 per cent alcohol until the flask is about half full and the sodium fluoride is brought down as a fine precipitate.

Dissolve 0.5 gm. of mercuric chloride in 3 c.c. of 95 per cent alcohol and add this to the fluoride suspension, making up the volume with additional alcohol.

The preservative thus prepared will show only a slight deposit of mercury and will be suitable for use for at least a month if kept in a dark cupboard.

PREPARATION OF THE PIPETTES

Shake the suspension thoroughly, then pour 2 or 3 c.c. into a small beaker. Agitate the beaker to make sure that the salt is uniformly dispersed and draw the liquid and suspension into the pipette to the second graduation. As many pipettes as desired may be filled at one time, the suspension in the beaker being stirred well with each pipette before it is filled and the pipettes laid horizontally after filling. Each pipette in turn is then tipped slightly toward the blunt end so that the suspension particles are drawn away from the tip, then tilted slowly toward the tip so that the liquid begins to flow away from the salt. When the liquid reaches the opening at the tip of the pipette apply a cloth or filter paper to draw it off, being careful not to absorb any of the salt. If the precipitate runs toward the tip too swiftly tilt once more toward the other end before continuing to decant at the tip. It is easier to see the precipitate and to regulate its progress if the pipette is held in front of some black surface as a background. When the salt is concentrated into a space a little less than a cm. in length at the tip of the pipette, direct a gentle current of air through the tip from a rubber tube. This will cause the wet salt to flow back along the bore of the pipette. Care must be taken to see that none is blown out the opposite end. Continue the gentle blowing until the salt is dried in a thin, even lining for the pipette. It requires only about a minute's time to line a pipette in this way, and once prepared and dried it may be kept indefinitely before using.

COLLECTION OF THE BLOOD

Make the lancet puncture in the finger-tip and collect the blood in the usual way for the micro method, allowing it to flow into the pipette to the second graduation and being careful not to admit bubbles. Then tip the blood column from one end to the other in the pipette, three times in each direction, in order to dissolve the preservative. Finally let the blood run down to the tip again, insert the tip of the pipette into a fold of the rubber band and stretch the band over the opposite end, pressing it down snugly over the blunt end. If the edges of the blunt end are not well covered some evaporation of the blood column toward that end may occur.

The bands should be washed with water and wiped dry before using.

If the first drop of blood fails to enter the pipette readily, wipe the tip with a cloth moistened in alcohol.

MAKING THE DETERMINATION ON PRESERVED BLOOD

Remove the rubber band and with a dampened cloth wipe off any blood on the outside of the pipette. Then with a slender wire such as the one inside a syringe needle, loosen the blood inside the tip. With the tip end held up, shake the pipette downward until the blood column begins to fall, then reverse the pipette and allow the blood to flow toward the tip again. Repeat this process until the blood cells, which may have settled along one side or at one end, are again mixed with the plasma. Then run the blood down to the tip once more, lower the meniscus to the 0.1 c.c. mark and blow the measured blood into the diluting fluid. Rinse the pipette repeatedly. Finish the sugar determination according to the Folin method.[1, 2, 3*]

*These methods have been summarized in a recent paper by Folin, New England J. Med. 206: 727, 1932. The sodium sulphate recommended in the precipitating reagent has not been used in our determinations since it was impossible to prevent hemolysis of the preserved blood samples.

The ease with which the blood begins to flow depends to some extent on the length of time since it was collected and also on the weather. The blood will remain more loose and fluid in the pipette at lower temperatures than during the very warm months.

In some cases it will be found that small patches of blood will adhere to the inside of the pipette and the rinsing is then very important. It will be found either to

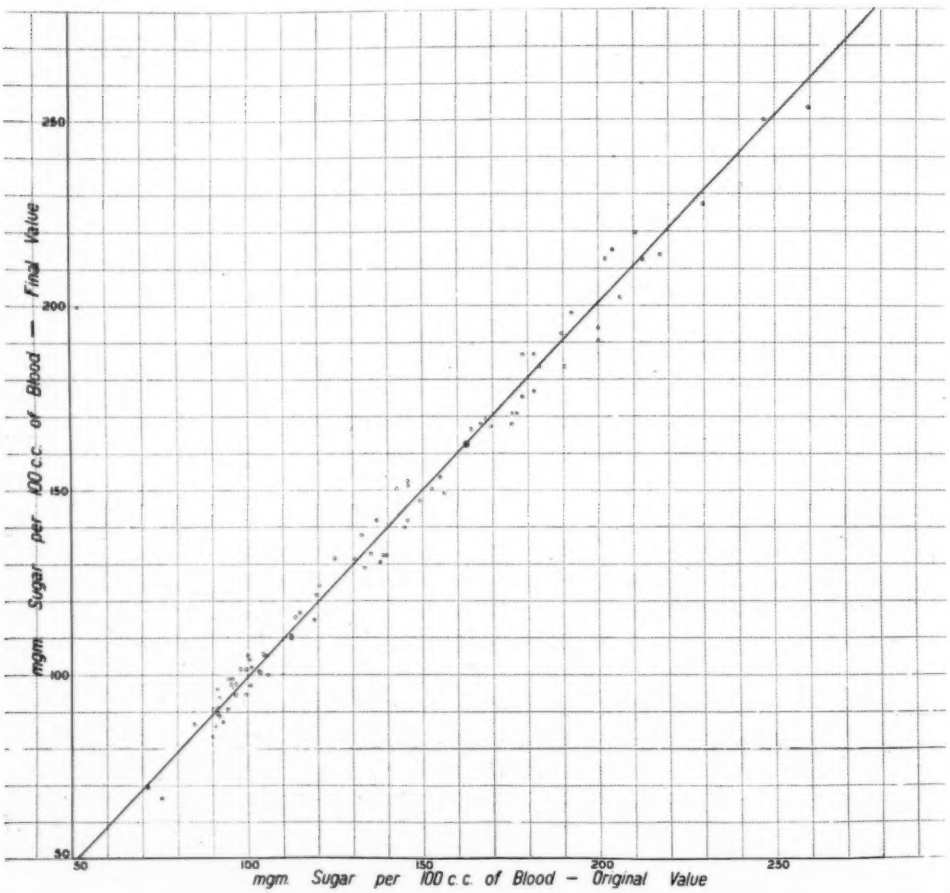

Fig. 1.—"The scatter diagram shows the results of the second test of each sample plotted in rectangular coordinates against those of the first. It is apparent that the scatter points fall rather closely around a line at forty-five degrees of the coordinate axes. The fact that the angle at which the line is inclined is forty-five degrees means that the first and second tests give, on an average, the same results; and the fact that the points cluster closely around the line is the expression of the fact that this relation is rather closely followed throughout the series of tests. The line has been laid by the method of least squares." (Alfred J. Lotka.)

dislodge the blood or to extract its glucose. A stiff piece of rubber tubing, 4 or 5 cm. in length, may be conveniently attached to the pipette to serve as a mouth piece for the rinsing.

EXPERIMENTAL RESULTS AND DISCUSSION

A series of blood specimens were kept from one to fourteen days and showed sometimes a slight drop, as often a slight increase over the corresponding fresh samples. The practice was to collect two samples of the same blood, one to be ana-

lyzed at once, the other to be kept in the pipette. Out of 88 comparisons made during the spring and summer of 1931, only 11 showed an error of more than 5 per cent. The greatest error encountered was 7.5 per cent. The figures for these comparisons are given in Table I. A graph has been prepared through the courtesy of Dr. Alfred J. Lotka to represent the facts brought out by the experimental data (Fig. 1).

TABLE I

A COMPARISON OF THE GLUCOSE VALUES OF FRESH SAMPLES AND PRESERVED SAMPLES OF THE SAME BLOODS

NO.	DAYS KEPT	GLUCOSE IN MILLIGRAMS PER CENT		PER CENT OF DEVIATION	NO.	DAYS KEPT	GLUCOSE IN MILLIGRAMS PER CENT		PER CENT OF DEVIATION
		FRESH SAMPLE	PRESERVED SAMPLE				FRESH SAMPLE	PRESERVED SAMPLE	
1	5	217.5*	213.9*	1.7	45	10	136.9	141.8	3.6
2	5	100.8*	97.1*	3.7	46	8	96.6	94.8	1.9
3	7	84.9*	86.8*	2.2	47	8	202.0	212.7	5.2
4	1	90.1*	90.9*	0.8	48	8	105.8	100.0	5.5
5	6	189.5*	192.4*	1.5	49	10	100.0	105.2	5.2
6	6	229.9	227.3*	1.1	50	7	137.9	130.7	5.2
7	6	94.6*	99.0	4.7	51	6	101.0	102.0	1.0
8	6	152.6	150.4	1.4	52	6	210.5	219.7	4.4
9	6	132.5*	137.9	4.1	53	5	163.9	166.6	1.6
10	6	98.0	101.5	3.5	54	11	94.3	90.9	3.6
11	6	206.1	202.0*	2.0	55	10	138.8	132.4	4.6
12	6	135.1*	132.9*	1.6	56	10	169.4	167.3	1.2
13	6	91.9*	93.9*	2.2	57	9	99.5	101.5	2.0
14	4	176.9	182.9	3.4	58	9	200.0	206.1	3.1
15	4	92.0*	95.1*	3.4	59	9	175.4	166.6	5.0
16	3	183.4	183.4*	0.0	60	9	162.5	162.5	0.0
17	6	168.0	169.4	0.8	61	9	104.7	105.2	0.5
18	6	96.6	97.6	1.0	62	6	204.0	215.0	4.9
19	6	142.7	150.3	5.3	63	10	119.7	121.8	1.8
20	6	90.1	83.3	7.5	64	10	113.6	115.6	1.8
21	6	120.4	124.2	3.2	65	6	112.3	110.5	1.6
22	6	178.5	175.4	1.7	66	6	90.5	90.1	0.4
23	6	200.0	190.4	4.8	67	6	156.2	149.2	4.5
24	6	133.3	129.0	3.2	68	6	190.4	183.4	3.7
25	4	241.0	232.5	3.5	69	8	103.1	101.0	2.0
26	6	181.8	176.9	2.7	70	12	104.1	105.8	1.6
27	6	145.9	141.8	2.8	71	11	192.4	198.0	2.9
28	12	95.7	99.0	3.5	72	7	139.8	132.4	5.3
29	6	90.9	86.2	5.2	73	5	91.3	96.1	5.3
30	6	145.9	151.4	3.8	74	6	155.0	153.7	0.8
31	6	130.7	131.5	0.6	75	6	91.3	89.7	1.8
32	6	114.9	116.9	1.7	76	6	91.3	91.3	0.0
33	1 hr.	71.6	69.7	2.7	77	6	145.9	152.6	4.6
34	6	95.2	97.6	2.5	78	5	99.5	94.8	4.7
35	10	124.9	131.5	5.3	79	10	181.8	186.9	2.8
36	8	105.2	105.2	0.0	80	6	175.4	168.0	4.2
37	8	212.7	212.7	0.0	81	6	266.7	274.0	2.7
38	8	247.0	250.0	2.0	82	6	303.0	317.5	4.7
39	8	175.4	170.9	2.6	83	6	259.7	253.1	2.5
40	10	119.0	114.9	3.4	84	10	112.3	109.8	2.2
41	9	149.2	147.0	1.5	85	7	166.6	168.0	0.8
42	11	93.0	87.3	6.1	86	9	144.9	139.8	3.5
43	8	100.5	104.1	3.6	87	7	103.6	100.5	3.0
44	8	178.5	186.9	4.7	88	4	162.5	162.5	0.0

*This figure is an average of duplicate determinations.

The keeping period is apparently limited only by the beginning of evaporation of the blood column. It is easy to tell by inspection if evaporation has occurred to any extent. If so the value will tend to be high. It has seldom been noted under eight days even in warm weather and showed only very slightly after fourteen days in the winter.

The procedure was adopted by this Company for tolerance tests in October, 1931, and has been used since then with acceptable results in all cases where the collection was made according to instructions. A grooved wooden box holding the pipettes, bands and a lancet is placed inside a pasteboard carton for mailing.* Six of these boxes, containing three samples each, were sent to San Francisco to be returned as a test of their travelling qualities. Four of the boxes showed the samples in as perfect condition on return as when mailed. In two of the boxes a slight separation in the blood column toward the blunt end of the pipette occurred. This

TABLE II

THE GLUCOSE VALUES OF BLOOD SAMPLES SENT THROUGH THE MAIL AND OF THE CORRESPONDING FRESH SAMPLES COLLECTED AT THE SAME TIME

BOX NO.	DATE SENT	DATE ANALYZED	GLUCOSE MILLIGRAMS PER CENT	
			MAILED SAMPLE	FRESH SAMPLE
1	Feb. 11	Feb. 19	92.6	97.6
			183.4	180.1
			66.8	68.5
2	Feb. 15	Feb. 29	129.0	129.8
			124.9	
			129.0	
3	Feb. 17	Feb. 29	90.9	94.8
			212.7	215.0
			92.6	100.0
4	Feb. 17	Feb. 29	155.0	157.4
			156.2	
			162.5	
5	Feb. 17	Feb. 29	88.9	88.9
			176.9	180.1
			50.3	56.3
6	Feb. 24	Mar. 4	91.7	90.9
			169.4	161.2
			107.0	110.5

did not interfere with the analysis, since the separated blood could be tipped out before the sample was measured. Results of the analyses of these samples are given in Table II.

Since the method has given acceptable results for glucose preservation, plans are now being made to adjust the procedure to the determination of urea.

*The outfits now in use were made especially for the purpose by the Empire Laboratory Supply Company, New York, and satisfactory rubber bands were obtained from the Hodgman Rubber Company, Malden, Mass.

SUMMARY

A practical method has been devised for collecting small samples of blood and preserving their glucose content. The samples are collected and kept in capillary pipettes sealed by means of rubber bands. As a preservative the pipettes contain a small amount of sodium fluoride and mercuric chloride, introduced in the form of a suspension-solution. Blood samples may be kept from one to fourteen days or until evaporation begins. In a large majority of cases the glucose value will remain constant within an error of 5 per cent.

The author is indebted for helpful advice to Dr. N. R. Blatherwick who suggested this problem and who helped to adapt the method to the purposes of the Company.

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A METHOD OF PREPARING BLOOD SMEARS*

KATSUJI KATO, M.D., CHICAGO, ILL.

NOTHING is more essential than the proper preparation of the smears in the routine examination of the blood, as well as in a study of the morphology of the blood cells. As even the best trained technicians in large hospitals pay little attention to the proper method of preparation, many blood smears are so poorly made that they prove to be wholly inadequate for even the ordinary differential count, not to speak of the more exacting cytologic study. The average clinicians who are frequently compelled to make the smears themselves are even more careless in this important procedure. I feel that this is not so much a matter of experience, as it is a matter of the use of a proper method.

In the literature on hematologic technic, I find that only the two most commonly employed methods are described.[1] They are (1) Ehrlich's two-cover-glass method, and (2) the two-slide method. Both of these time-honored methods are unsatisfactory. The first of these gives a fairly good result in the hands of experienced persons, but it has many disadvantages. The cover glasses are thin and small, causing much difficulty in manipulation and often loss of the specimen due to breakage. The two-slide method is utterly defective in its fundamental principle, as recently brought out by Silberstern.[2] By this method the distribution of the leucocytes is greatly distorted or they are even pushed away to the edges of the slide. The painstaking work of Gyllenswärd[3] only emphasizes the undersirability of the old methods.

The method to be described here was shown me by Dr. William Bloom in the Department of Anatomy of the University of Chicago, who developed this technic. Dr. Bloom tells me that he is not aware of its being used by anyone else, but that he is unwilling to claim the method as his own, for the procedure is so simple that it must have occurred to other workers with the dry smear technic.

*From the Department of Pediatrics, the University of Chicago.
Received for publication, May 19, 1932.

Although I have never seen this technic used by others, I think it desirable to report this method because it is a very convenient means for obtaining a uniform distribution of the cellular elements of the blood.

A clean slide is used to receive a drop of blood flowing from a fresh stab wound. The drop should not be too large, the optimum size being from 3 to 5 mm. in diameter when received on the slide. The initial position of the drop on the slide is of some importance. It should be slightly to one side away from the center of the slide. An oblong cover glass, 24 by 50 mm. is laid immediately over the drop-

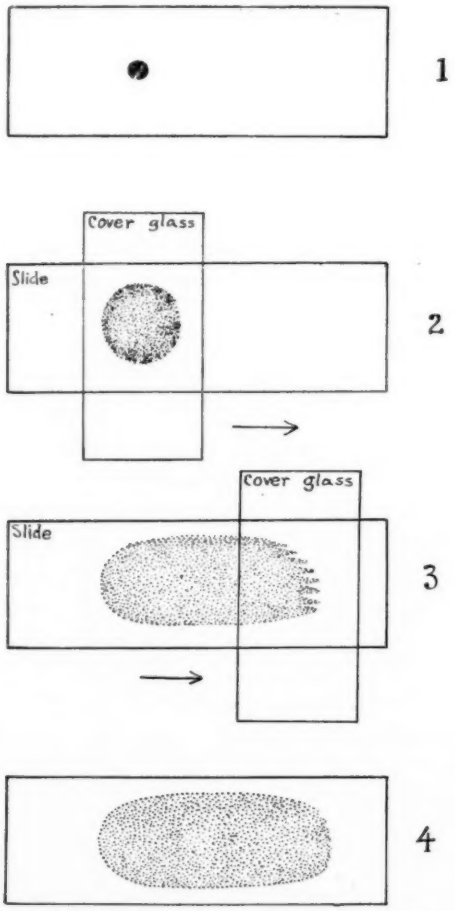

Fig. 1.—Showing steps of preparing blood smear.

let and the blood is allowed to spread between the cover glass and the slide into a circle of thin film by the weight of the cover glass. No pressure should be exerted over the cover glass for this purpose. When the blood has spread into a circle of appropriate dimension, the cover glass is quickly drawn across toward the other end of the slide, as illustrated in Fig 1. This will give an elongated oval of thin film, the border of which should be well within the edges of the slide. The slide should be fanned at once and air dried in the usual manner. The film so prepared will be found to have an even distribution of the leucocytes in almost every microscopic field. The erythrocytes will also be found to be spread in a single layer without overlapping or rouleaux formation.

The thickness of the film may be easily controlled, after a little experience, by the length of time which is allowed to elapse from the moment the cover glass is applied until it is pulled across. As a rule, the longer the time, the thinner the film, and vice versa. The blood of a leucemic patient will have to be spread rather thinly, as the leucocytes are usually numerous, while leucopenic blood should be spread thickly. Viscocity of the blood will also affect the thickness of the film. It is difficult, for example, to make a thin film from the blood of a newborn infant due to its greater viscosity. It will be found that if the cover glass is allowed to remain too long, it will adhere to the slide, causing difficulty in further manipulation. The plethoric or hydremic blood often found in leucemias and certain other blood dyscrasias needs to be worked with moderate speed, or the film will tend to become too thin. The blood of certain mammals, the reptiles and the amphibians, containing very large corpuscles, must be spread rather thick, commensurate with the greater diameter of the blood cells. Each worker will have to find out the finer details and particulars from personal experience.

This method is made public for two reasons: (1) Because the literature on hematology commonly consulted by both clinicians and technicians fails to mention this method, and (2) because it gives uniformly good blood smears.

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AN AIR MOTOR FOR STIRRING*

ARLINGTON C. KRAUSE, PH.D., M.D., BALTIMORE, MD.

THE following air motor was devised as a substitute for the electric motor. Fluctuation of the direct current of the clinical laboratory caused a racing of the electrical stirring apparatus, which resulted in the destruction of glass beakers and other vessels. In use, the air motor has demonstrated many advantages over the electric motor. It has been found to be light in weight, quickly adjustable to any angle, cheaply made, and vibrationless. It takes a small amount of air and may be run constantly day and night with little or no care.

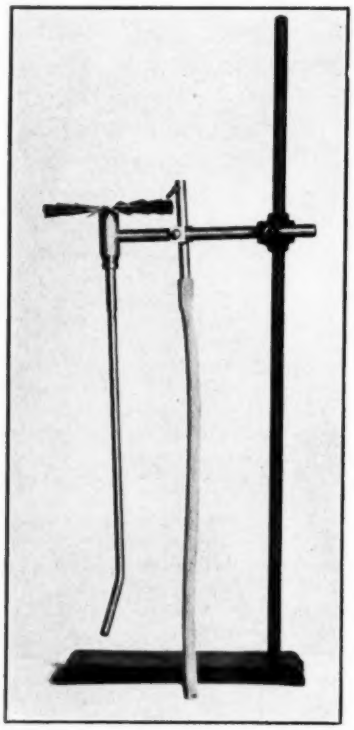

Fig. 1.

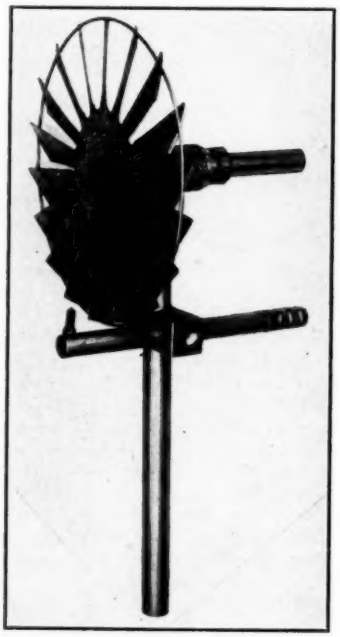

Fig. 2.

APPARATUS

A 4-inch fan was made from a circular piece of No. 24 gauge brass plate, cut radially 1¼ inches to make 18 blades. The blades were turned 30°, and fastened to a circular brass wire for stability. The fan was soldered onto a fixed brass collar, which was attached to a brass shaft 3 inches long and ¼ inch in diameter.

*From the Wilmer Ophthalmological Institute of the Johns Hopkins Hospital and University.

Received for publication, May 21, 1932.

Directly below the collar, the shaft was passed through the vertical part of a T-shaped brass holder, which was used as a journal. A hole was bored into the side of the journal for the purpose of oiling. The horizontal part of the T-shaped holder acted as a side arm. Another collar, attached to the shaft by a setscrew, was placed below the holder to keep the shaft in place. The lower end of the shaft was made into a female screw with a beveled shoulder to hold stirring rods and other attachments. Holes were drilled transversely through the shaft and through the stirring rods to aid in disconnecting them. The motor described above was operated by means of compressed air which was passed through a rubber hose into a vertical brass tube, which was attached by a setscrew to the side arm of the holder. The air was then directed over the fan through a 1/16 in. air nozzle, which was set into the side of the tube at a 45° angle. This air motor was supported by clamping the side arm to a laboratory stand.

The stirring rods were made of brass and monel metal with male screw ends. A glass stirring rod was constructed by cementing a glass rod into the hollow tip of a short metal rod having a male screw end. A flask, attached to a straight stirring rod by means of a rubber stopper and then rotated by the motor, was also used for agitation. Large flasks were easily turned in a water bath because of the buoyancy of the water.

The air motor described above was an improved design of another model which was easily constructed. The fan was constructed from the top of a discarded ether can. Blades of the fan were made by cutting the top peripherally in radial sections 5 cm. deep and 1 cm. wide. The blades were turned 45° by means of pliers. A one-holed rubber stopper holding a glass stirring rod was placed in the central opening of the fan. A journal for the stirring rod was made from a one-holed rubber stopper, which held a piece of glass tubing. The journal was supported by a laboratory clamp. Compressed air from a glass nozzle revolved the fan. This simple type of air motor was useful for many operations for which an electric motor was too expensive.

THE CALIBRATION OF MICROPIPETTES BY A COLORIMETRIC METHOD*

FRANKLIN C. BING, PH.D., CLEVELAND, OHIO

THE increased use of micro methods of blood analysis necessitates a fairly rapid and convenient technic for testing the accuracy of the small pipettes used in such work. Calibration by the classical method, weighing the amount of mercury delivered, is often a tedious process and may be attended with several sources of error that are obviated only by taking unusual care. During the past two years we have used a simple colorimetric procedure for the calibration of pipettes having capacities varying between 0.0005 c.c. and 1.000 c.c. This method possesses the theoretical advantage that the calibration is performed with whole blood, the fluid which most of the pipettes are intended to measure. With the gravimetric procedure, it is frequently necessary to allow a correction for the difference in volume between a meniscus formed by mercury and that formed by water or blood. The colorimetric method automatically eliminates such calculations.

METHOD

Several cubic centimeters of fresh oxalated or defibrinated blood are obtained. From the well mixed specimen a sample is removed with a pipette calibrated by standard methods to contain 1 c.c. The blood is allowed to run into a clean and empty 2 liter flask, the tip of the pipette being held against the glass. The blood in the neck of the flask is washed down with a few cubic centimeters of distilled water. The pipette is rinsed into a beaker, and the washings quantitatively transferred to the flask. In adding water to the flask it should be allowed to run down the sides to avoid the formation of foam. Dilution is made to the mark and the contents are thoroughly mixed. Using calibrated apparatus, this process yields a standard blood solution of accurately known dilution.

The pipette to be calibrated is carefully cleaned, dried, and filled to the mark with the same blood from which the standard solution is prepared. The pipette should be calibrated in precisely the same way as it is to be used, either *to deliver* or *to contain,* the latter method being preferred. The blood is diluted with sufficient water to make the final volume about 2000 times the nominal capacity of the pipette. Thus, 0.1 c.c. of blood is diluted to 200 c.c., using a calibrated volumetric flask, and 0.001 c.c. of blood is transferred into 2 c.c. of water, measured with an accurate pipette into a clean, dry test tube. With volumes smaller than 0.001 c.c., the approximate dilution factor of 2000 is neglected, the final volume being maintained at 2 c.c. because 1 c.c. is required for the colorimetric determination. There is thus obtained a blood solution of which, in all cases, the final volume is known but the exact dilution is unknown.

*From the Department of Biochemistry, School of Medicine, Western Reserve University.
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Hemoglobin estimations are made upon both samples of diluted blood by the benzidine method.[1, 2] The colors produced are compared in a colorimeter.

CALCULATIONS

The actual capacity of the pipette is obtained by substituting the proper numerical values in the following equation:

$$C = \frac{S \times v_1 \times b}{R \times v_2}, \text{ in which}$$

C = the capacity of the pipette
S = the colorimeter reading of the blood used as standard
R = the colorimeter reading of the blood of unknown dilution
v_1 = the total volume of diluted blood of unknown dilution
v_2 = the total volume of diluted blood used as standard
b = the actual volume of whole blood employed in preparing the standard dilute blood

In practice, the values for S and R are best determined by triplicate or quadruplicate determinations. To illustrate by an actual example: a pipette supposed to hold 0.020 c.c. is calibrated, the final volume of the diluted blood being 50 c.c., and the standard consisting of 1.005 c.c. of blood diluted to 2000 c.c. The observed colorimeter readings of three standard tubes, the first being set at 10 mm., are 10, 10, and 10 mm. Three tubes prepared from the blood of unknown dilution give readings of 12.2, 12.2, and 12.3, average 12.2 mm. The capacity of the pipette is calculated as follows:

$$C = \frac{10.0 \text{ mm.} \times 50.00 \text{ c.c.} \times 1.005 \text{ c.c.}}{12.2 \text{ mm.} \times 2000 \text{ c.c.}}$$
$$= 0.0206 \text{ c.c.}$$

ACCURACY OF THE METHOD

In Table I are presented data comparing the capacities of several pipettes as determined by the present method and by means of the usual procedure, weighing mercury. In each case, the figures in the table represent either averages obtained

TABLE I

COMPARISON OF CALIBRATIONS WITH COLORIMETRIC METHOD AND WITH MERCURY

PIPETTE NO.	COLORIMETRIC METHOD C.C.	GRAVIMETRIC METHOD C.C.
1	1.000	1.005
2	0.1104	0.1096
3	0.1022	0.1011
4	0.0262	0.0262
5	0.00664	0.00662
6	0.00571	0.00572

from colorimetric readings in triplicate or quadruplicate on a single dilution of blood, or the average of two weighings of mercury. It is apparent that the differences in the values obtained by the two methods are not more than 1 per cent, which is entirely satisfactory for many practical purposes.

An independent check on the accuracy of the calibration is afforded by com-

parison of the colorimetric hemoglobin estimation of a second blood sample with the value obtained by the oxygen capacity method of Van Slyke and Neill.[5]

Duplicate calibrations by the colorimetric method have yielded values that differ from each other by not more than 1 per cent. With pipettes having capacities less than 0.001 c.c., however, it is difficult to attain this order of accuracy unless the size of the capillary is so chosen that the length is sufficient to minimize the error of reading the meniscus. It is obvious that the accuracy of the method renders it of no special value, except, perhaps, that of convenience, for the calibration of pipettes having capacities greater than 0.1 c.c. It has, however, been satisfactorily employed in standardizing micropipettes intended for the determination of hemoglobin, sugar, and other constituents in blood.

APPLICATION TO THE CALIBRATION OF DILUTION PIPETTES USED IN THE ENUMERATION OF BLOOD CELLS

The value of the present method is revealed by the ease with which the dilution pipettes used in hemacytometry can be calibrated. Even though the bulb of such pipettes may have a volume considerably greater than 0.1 c.c., calibration by the colorimetric method has proved more reliable than the gravimetric method in our hands, due to the difficulty encountered in trying to fill the pipette. This is partly due to the fact that the glass bead present in the ampulla floats on the mercury.

These pipettes can also be calibrated by weighing the amount of water which they remove from a partly filled and counterpoised weighing bottle. The U. S. Bureau of Standards tests the calibration of dilution pipettes by a volumetric method with special apparatus.[4] It may be noted that the allowable error of the dilution ratios, according to the present specifications of the Bureau,[3] is, for white cell pipettes, ±3.5 per cent, and for red cell pipettes, ±5 per cent. In other words, if the erythrocytes are enumerated in a blood specimen having a true count of 5.00 million per cubic millimeter, the value obtained with certified pipettes may be anywhere between 4.75 million and 5.25 million, inclusive, omitting all errors except those of the measuring pipettes. While such variations would never lead to erroneous clinical interpretations, yet, for accurate experimental work, we have found it necessary to calibrate all red cell dilution pipettes at the 0.5, 1.0, and 101 marks. For this purpose the present method has proved entirely satisfactory.

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THE COMPARATIVE EFFICIENCY OF THREE STAINS FOR TUBERCLE BACILLI*

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IT IS a well recognized fact among laboratory workers that various acid fast stains vary in efficiency for the routine examination of sputum for tubercle bacilli. Also various modifications of the same stain vary in results. Brown and Hayes[1] found that the use of brilliant green instead of methylene blue gave an increase of 8 per cent in positive findings.

Furthermore, it is recognized that at various stages of growth the tubercle bacillus varies in its acid fast quality, depending on the media and length of time it is grown on the same media.[2]

There are nonacid fast, weakly acid fast, and strongly staining acid fast bacilli.

In this Hospital it has been the custom to use these stains: Ziehl-Neelsen, Schulte-Tigges,[3] and Spengler.[4] These stains are all used on the same specimen of sputum.

The technic as we do them are as follows:

1. Ziehl-Neelsen:
Steam with carbol-fuchsin five minutes.
Wash with water.
Decolorize in acid alcohol eight minutes.
Wash with water.
Counter stain thirty seconds with 1 per cent methylene blue.
2. Schulte-Tigges:
Steam with carbol-fuchsin one minute.
Wash with water.
Decolorize with 10 per cent aqueous solution of sodium sulphite (until color practically disappears).
Wash with water.
Counter stain with saturated aqueous solution of picric acid.
Wash with water, dry, and examine.
By this method the bacilli are red against a yellow background.
3. Spengler:
Stain with carbol-fuchsin (warm but not hot enough to bubble) five minutes.
Pour off stain and without washing.
Pour on picric acid alcohol four to five seconds (1 part saturated solution aqueous picric acid 1 part 95 per cent alcohol).
Wash with 60 per cent alcohol.
Treat with 15 per cent nitric acid till yellow (not over thirty seconds).
Counter stain with picric acid alcohol solution until lemon yellow.
Wash with distilled water, dry, and examine.
Bacilli are brownish-red against a yellow background.

These statistics comprise the results of several years' work, and were performed through curiosity and without prejudice in favor of any stain.

*From the Woodmen of the World Hospital.
Received for publication, May 27, 1932.

Each slide was examined in ten random fields except where such fields were negative, in which case a thorough search of the entire slide was instituted.

As a majority of our cases are far advanced the numbers of bacilli are more abundant than would be found in institutions limiting patients to first and second stage cases.

There are, however, enough cases in which bacilli are absent or few to evaluate the tests in cases with but few bacilli. We also feel the series is sufficiently large to compensate for any errors due to the human element, variations in thickness of smear, etc.

TABLE OF EXAMINATIONS

Cases examined	600
Slides examined 3 by 600	1,800
Fields examined 10 by 1,800	18,000
Total Number of Bacilli Counted:	
1. Ziehl-Neelsen	19,692
2. Schulte-Tigges	51,759
3. Spengler	71,559
Total	143,010

Grossly, it will be seen that the Schulte-Tigges is almost three times as efficient as the Ziehl-Neelsen, and the Spengler almost four times as efficient. This does not, however, prove the latter two stains are worth the extra trouble of using them, as the vast majority of slides showed abundant numbers of bacilli with any of the three stains.

Therefore, a comparison of the negative slides is in order.

1. Ziehl-Neelsen	166
2. Schulte-Tigges	144
3. Spengler	140

It would appear then that by using Spengler's method one would get twenty-six positives for every one hundred sixty-six negative slides by the Ziehl-Neelsen method—an advantage of 12 per cent.

The advantage of Spengler's method in facilitating search for the bacilli is further shown by the statistics of negative fields in slides in which bacilli were somewhere found.

	Ziehl-Neelsen	Schulte-Tigges	Spengler
Fields negative in positive slides	427	137	104
To which should be added 10 fields for totally negative slides	1660	1440	1440
Total	2087	1577	1504

Thus, out of 2087 negative Ziehl-Neelsen fields Spengler's method showed 2087-1504 or 583 positive fields, an advantage of over 25 per cent.

As can readily be seen the Schulte-Tigges is but slightly less efficient than the Spengler. However, it is a much more difficult stain to perform and the chance of error in technic is large. The sodium sulphite must be absolutely fresh, and must not be used for more than a very few slides before being changed.

It will be argued that the Ziehl-Neelsen is the better stain for the routine examination of sputum by nonlaboratory men. Undoubtedly it and its modifica-

tions have stood the test of time against many newer methods. Certainly it is more difficult to over decolorize with acid-alcohol than with nitric acid. However, the use of nitric acid is for so short a time (thirty seconds) that it can readily be timed by the watch accurately and without in any way being onerous. Whereas, the acid-alcohol method requires considerable waiting. We feel that students with a little practice could do this stain well and save time.

Following the technic in use here it takes approximately sixteen minutes to do a Ziehl-Neelsen stain and seven minutes to do a Spengler. The saving of time is considerable. Where a Ziehl-Neelsen technic using acid and then alcohol is employed the time consumed would be approximately the same in either case.

CONCLUSIONS

1. It is evident that in old chronic third stage cases expectorating large numbers of bacilli that a far greater percentage of bacilli have to a certain extent lost some of their acid fastness (and probably some are completely nonacid fast), as compared with cases of but slight involvement expectorating smaller numbers of bacilli. The comparison of the total Ziehl-Neelsen (19,693 bacilli) with the total Spengler (71,559 bacilli) adequately illustrates this point.

2. That Spengler is a better stain from standpoint of actual results in showing the presence of tubercle bacilli, is adequately shown by these statistics.

3. The Ziehl-Neelsen technic has no advantage in respect to the time saving element, and if acid-alcohol is used is actually much more tedious.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

HYDATID MOLE: A New Method of Diagnosis and Prognosis, Mayes, B. M. J. Australia II: 20: 603, 1932.

The author calls attention to the fact that in the presence of a hydatid mole the urine contains enormous quantities of anterior pituitary hormone (200,000 to 500,000 mouse units per liter).

The following procedure is based upon this fact.

A morning specimen of urine is diluted with distilled water 1:50 in a test tube and 1:100 in another test tube.

An Aschheim-Zondek test is done with each dilution and also with the undiluted urine.

If the reaction is positive with the undiluted urine alone the case is one of pregnancy, but if positive with either of the above dilutions, the reaction is interpreted as positive for hydatid mole.

POLIOMYELITIS: Resistance to, Jungeblut, C. W., and Engle, E. T. J. A. M. A. 99: 2091, 1932.

1. The widely accepted hypothesis which postulates that the resistance of adults to poliomyelitis is due exclusively to universal subclinical immunization against the virus is inconsistent with a number of epidemiologic observations bearing on the age distribution in various latitudes and the recent trend towards an advance in the age line.

2. The assumption of a unique immunizing power of the virus in the absence of disease production cannot be verified experimentally.

3. The opinion that natural virucidal substances in the serums from human adults can develop only through previous contact with the specific antigen is open to criticism because of their apparent formation in man and monkeys under conditions which preclude the presence of the virus.

4. The evidence in support of the current concept that the incidence of virucidal serums in human adults stands in direct relation to the density of population should be reviewed since individual serums may show wide discrepancies in virucidal power, which seems to vary with the blood group and to fluctuate with changes in the physiologic state of the body.

5. In a restricted number of instances it has been possible to demonstrate that virus-neutralizing substances may occur in the serum of immature monkeys after prolonged treatment with anterior pituitary extracts or similar principles recovered from the urine of pregnant women.

6. The suggestion is offered that the mass protection enjoyed by the adult human population rests primarily on the normal function of the endocrine balance characteristic of mature age.

Urinary Antisepsis, Davis, E., and Sharpe, J. C. J. A. M. A. 99: 2097, 1932.

As determined by antiseptic tests made before and after administration, pyridium, given in capsules to normal persons in unit maximum dosage (0.4 gm.), colors the urine.

Caprokol (dose, 0.75 gm.) judged by the same standard, exerts an irregular and transient antiseptic action in about one-third of the four hour samples.

Methenamine (dose, 1 gm.) is incomparably more efficient than either pyridium or caprokol in causing the normal person to secrete urine which is antiseptic against both the colon bacillus and the staphylococcus.

Acriflavine (dose, 0.2 gm.) administered in capsules, exerts an antiseptic action in normal urine against both the colon bacillus and the staphylococcus, which is uniform and consistent to a surprising degree. Urinary alkalinity is essential.

Acriflavine (dose, 0.2 gm.) administered in shellac-covered pills, is practically inert.

Acriflavine, administered in capsules, although noninjurious in efficient dosage, causes unpleasant symptoms (nausea and catharsis) in a fair proportion of cases.

Acriflavine, for the reason given, has its definite clinical limitations. Clinical experience indicates, however, that this drug is of distinct value in selected cases, particularly those of acute infections of the urinary tract.

LEPROSY: The Demonstration of in the Blood by Means of the Thick Drop Method, Sardjito, M., and Sitanala, J. B. Med. Dienst. d. Valksgez in Nederl.-Indie 21: 32, 1932.

The authors confirm the demonstration of lepra bacilli in the blood by the thick drop method as used for malaria, using, of course, the Ziehl-Neelsen stain.

B. C. G.: A Study of the Pathogenicity of, Feldman, W. H. Am. J. Path. 8: 755, 1932.

Using a strain of B. C. G. obtained from Calmette of the Pasteur Institute, a delibérate attempt was made to increase its pathogenicity by subculturing the organism on a glycerinated egg medium. Transfers were made every thirty days. From each succeeding subculture 4 guinea pigs were given injections, 2 intracerebrally, 1 subcutaneously, and 1 intraperitoneally. The report deals with data obtained after the organism had been subcultured on glycerinated egg medium for fifteen generations.

Of a total of 58 guinea pigs inoculated, lesions histologically indistinguishable from those of genuine tuberculosis occurred in the tissues of 11, and cultures of acid-fast bacilli were obtained from each. Although the majority of the lesions occurred in animals that had been given intracerebral injections, 1 animal that was given an intraperitoneal injection and another given a subcutaneous injection died with lesions of a tuberculous nature. So far, attempts have failed to promote a succession of tuberculous lesions by the reinoculation into guinea pigs of infective material from lesions.

The particular strain B. C. G. studied is not devoid of pathogenicity for guinea pigs, and the assertion that the organism is innocuous cannot be accepted without reservations.

Subculturing the organism on glycerinated egg medium at monthly intervals for a period of fifteen generations did not markedly enhance its virulence.

TISSUE STAIN: Silver Impregnation of Glia and Nerve Fibres in Paraffin Sections After Formalin Fixation, Wilder, H. C. Am. J. Path. 8: 785, 1932.

The following method has been found satisfactory:

Fixation and Embedding: Fix tissues in 10 per cent formalin, wash in tap water, dehydrate in alcohol, clear in chloroform, and embed in paraffin.

Bromuration: Pass paraffin sections through xylol and graded alcohols, rinse in distilled water, and place in 34 per cent hydrobromic acid for thirty minutes.

Sensitization: Wash in distilled water ten to twenty seconds and flood the slide with 1 per cent uranium nitrate (sodium free) for 5 seconds or less.

Impregnation: Wash in distilled water ten to twenty seconds and place for twenty seconds in silver diamino hydroxide:

To 5 c.c. of 10.2 per cent silver nitrate add ammonium hydroxide drop by drop until the precipitate which forms is dissolved. Add 5 c.c. of 3.1 per cent sodium hydroxide and just dissolve the resulting precipitate with a few drops of ammonium hydroxide. Make the solution up to 50 c.c. with distilled water.

Reduction: Wash in distilled water two seconds and agitate each slide separately in the following reducing solution until it ceases to give off a brown cloud:

Distilled water 50 c.c., 40 per cent neutral formalin (neutralized with magnesium carbonate) 0.5 c.c., 1 per cent uranium nitrate 1.5 c.c.

Counterstaining and Mounting: Wash in distilled water counterstain with eosin, dehydrate in alcohol, clear in xylol and mount in Canada balsam. Argentation frequently allows hematoxylin to be used as a nuclear stain, but bluing must take place in tap water, as ammonia dissolves the silver.

Distilled water is used in the preparation of all solutions. The uranium nitrate solution and the 10.2 per cent silver nitrate keep indefinitely and the silver diamino hydroxide keeps for a week or more in amber, glass-stoppered bottles. The impregnating and reducing solutions retain their activity in Coplin jars for two days. The hydrobromic acid may be kept in a Coplin jar and used repeatedly for an indefinite time. It is important that the ammonium hydroxide be kept in a well stoppered bottle.

ALLERGY, Value of Cytologic Examination of the Nasal Smear in Differential Diagnosis of Infection and, Kahn, I. S., and Stout, B. F. J. A. M. A. **99**: 1494, 1932.

The following conclusions are presented:

1. A positive eosinophilic nasal smear of from 10 to 90 per cent is almost invariably diagnostic of allergic rhinitis.

2. A positive eosinophilic smear of 4 per cent or over is highly suggestive of allergic rhinitis.

3. Conditions such as a pure, thin, watery discharge, allergic quiescence and, at times intercurrent infections in previously definitely proved cases of allergic rhinitis are proof that the absence of eosinophilia at a single examination does not bar allergic rhinitis.

4. The presence of abundant noneosinophilic polymorphonuclear leucocytes in the smear is practically diagnostic of infection.

5. Allergic and infectious rhinitis and sinusitis may exist coincidently or at alternate periods in the same person.

6. This simple nasal smear test, if done as a routine measure, will prove of decided diagnostic value to rhinologists and to workers on allergy.

LEUKEMIA, Some Immunologic Aspects of, Hulper, W. C., and Russell, M. Arch. Int. Med. **49**: 113, 1932.

This study was undertaken to test the theory that the increase of leucocytes in leucomias might be referable to a disturbance in the antiproliferative activity of the blood serum. The following conclusions are advanced:

1. The antiproliferative quality of leucemic plasma is not decreased, but slightly increased, as compared with that of normal human plasma. Myeloid leukemia is therefore not the result of the decrease or absence of this quality of the serum.

2. Myeloid leucemic plasma inhibits the emigration of the granulocytic elements from the explanted clot of blood leucocytes.

3. Antileucocytic serum produced by the intravenous injection of leucemic leucocytes into rabbits impairs the emigrative and proliferative qualities of normal and leucemic leucocytes in tissue cultures.

4. Tissue cultures of leucocytes allow a rather accurate titration of the cytotoxic qualities of an antileucocytic plasma.

5. Isolated favorable therapeutic results (in 4 of 11 cases) with nontitrated antileucocytic serum in leucemias seem to indicate that properly prepared and titrated antileucocytic serums may offer an effective remissive method for the treatment in leucemias.

CANCER, Interrelations Between Histologic Structure and Blood Chemical Findings in, Hulper, W. C., Woodward, G. E., and Fry, E. G. Am. J. Cancer **15**: 2666, 1932.

1. The malignancy indices and the blood P_H values of 34 cases of carcinoma were correlated and a preponderance of histologically highly malignant tumors was found in patients with an alkalosis, while the tumors of histologically low malignancy predominated in patients with a normal blood P_H.

2. The alkalosis of the blood in cancer patients varies not only with the extent of the disease, but also with the degree of malignancy and maturity of the tumor.

3. The degree of cellular irregularity of cancerous tissue is increased in patients with an alkaline P_H of the blood as compared with patients with a blood P_H in the normal range.

4. In about one-third of the cancer patients with a hyperglycemia (which was present in 78.3 per cent of our cases) a high number of mitoses was found, while this phenomenon was absent in cancers with a normal blood sugar.

AUTOHEMAGGLUTINATION, Hyperproteinemia as a Cause of, Reimann, H. A. J. A. M. A. **99**: 1411, 1932.

In a case of myeloma without Bence-Jones proteinuria, the clue to the underlying condition was obtained by an inability to count the erythrocytes because of immediate rouleau formation. As a result of this phenomenon, difficulties were encountered in preparing blood smears and in selecting a suitable donor for blood transfusion. Similar phenomena are encountered in other conditions associated with hyperproteinemia, as in pneumonia, kala-azar and pregnancy. Marked hyperproteinemia was present in the case of myeloma here reported. It is suggested that, in this case, disease of the bone marrow may be largely responsible for the increase of globulin and fibrinogen.

WATER: Study of Bacteriological Methods of Testing and Means of Disinfecting Water With Chlorine With Particular Reference to Swimming Pool Water, Mallmann, W. L., and Carey, W. Am. J. Pub. Health **23**: 35, 1933.

The authors report that samples of swimming pool water collected during periods of use and tested immediately showed more pollution than duplicate samples handled in the usual manner by storing and testing later.

During periods of use, swimming pool water showed marked pollution as measured by colon bacilli and streptococci indices in the presence of residual chlorine contents of 0.2 to 0.5 p.p.m.

During periods of rest, the pollution evidenced during use, disappeared. The rate of disappearance depended upon the type of treatment used.

With chloramine treatment, a delayed germicidal action occurred. This was also true of chlorine treatment in alkaline water, but to a lesser extent.

The colon bacilli and streptococci indices roughly parallel each other. The preponderance of the colon bacilli and streptococci incidences varies.

A sodium thiosulphate treated sample bottle is recommended for collecting pool samples.

The method advised for collection of samples follows:

The bottle is prepared by adding a small crystal of sodium thiosulphate to a moist bottle or 0.5 c.c. of tenth normal sodium thiosulphate to a dry bottle subsequent to sterilization by moist heat. Care must be taken not to rinse the bottle in collecting the sample. The sodium thiosulphate added is sufficient to neutralize a residual chlorine content of at least 1 p.p.m. The results obtained from sodium thiosulphate treated bottles and from poolside tested samples are similar.

TYPHUS FEVER, Laboratory Diagnosis of Endemic and Rocky Mountain Spotted Fever, Badger, L. F. Am. J. Pub. Health **23**: 19, 1933.

There are certain and definite criteria for the identification of an unknown strain of virus as being one of endemic typhus or spotted fever.

With the exception of the involvement of the external genitalia of the male guinea pig the clinical symptoms produced in laboratory animals by the virus of endemic typhus exhibit nothing characteristic of the disease. In the male guinea pig the western virulent virus of spotted fever produces a characteristic involvement of the external genitalia, while the viruses of the eastern spotted fever studied produce no symptoms in the guinea pig which are not produced by some known bacteria. Both the western virulent virus and the eastern virus of spotted fever may produce in the rabbit the characteristic involvement of the external genitalia and in the monkey the characteristic exanthem.

The viruses of spotted fever, as well as the virus of endemic typhus, produce in the brain of the guinea pig the nodal and vascular lesions seen in European louse-borne typhus.

The Weil-Felix reaction has been found positive with approximately 75 per cent of the sera of rabbits and monkeys inoculated with either the endemic typhus virus or the western virulent spotted fever virus. The Weil-Felix reaction has been found positive with but approximately 25 per cent of the sera of monkeys inoculated with the eastern spotted fever virus.

Immunity tests must be clean cut and made in both directions.

Cultures of the cardiac blood of animals from which transfers are made must be con-

sistently negative, as confusing clinical symptoms and immunity tests have been caused by known bacteria.

PREGNANCY, New Hormonal Reaction in, Hofmann, H. Zentralbl. f. Gynäk. **56**: 2534, 1932.

The author advocates the method following:

Withdraw 25 c.c. of blood by venipuncture, separate the serum, shake it with ether. The ovaries of a not less than a 2,300 gm. rabbit are inspected by a laparotomy and 13 c.c. of serum injected intravenously.

The ovaries are again inspected in twenty-four hours for the presence of hemorrhagic spots which indicate a positive reaction.

TUBERCLE BACILLI, Concentration of in Sputum, Nassi, E. Policlinico **39**: 1649, 1932.

Reagent: Magnesium hydrochloride 2 gm., sodium bicarbonate 6 gm., distilled water 1000 c.c.

Method: Mix equal parts of sputum and reagent and stir slowly with a glass rod for 10 minutes. Transfer to a large test tube and, while slowly stirring, heat gradually in a water-bath at 85 to 90° C.

Pour in a sedimentation cup (or centrifuge). After three hours (if sedimented), pour off the supernatant liquid and make smears from the sediment. Stain as usual.

A second rapid method is thus described:

Prepare a 15 per cent solution of gelatin and filter while hot.

Heat the sputum fifteen minutes in a water bath and when it is a homogeneous mass, to 10 c.c. of melted gelatin add 5 c.c. of heated sputum and 5 c.c. of ligroin in a test tube. Cork the tube and shake thoroughly for thirty seconds.

Place the tube in cold water until the gelatin solidifies. Then pour off the ligroin, scrape the surface of the gelatin and use the scrapings for smears.

The author recommends 4 per cent sulphuric acid for decolorization and well diluted methylene blue for counterstaining.

TUBERCULOSIS, Costa and Red Cell Sedimentation in Laryngeal, Rubinstein, C. Am. Rev. Tub. **27**: 92, 1933.

An evaluation of the Costa reaction and a comparison with the red-cell sedimentation reaction are presented, the material used for this purpose being 75 cases of pulmonary tuberculosis complicated by laryngeal tuberculosis.

Before instituting any radical form of treatment it is highly important that the phthisio-laryngologist secure accurate data regarding the patient's pulmonary process and his immuno-biological status.

The author recommends as a determinant of pulmonary activity, in addition to sedimentation reaction, the Costa reaction.

The Costa and sedimentation reactions disclosed a complete parallelism in cases of far-advanced disease. In latent cases and those with moderately advanced disease there was a disparity between the two reactions that favored the Costa in sensitivity.

Both reactions were found to be of value in differential diagnosis between simple and tuberculous laryngitis.

Tuberculosis of the larynx widens the indications for chest surgery which, when applied at the proper immunobiological phase, is a most powerful weapon in effecting a complete cure of laryngeal tuberculosis.

It is the author's opinion that the Costa reaction may be employed profitably in the laryngeal-tuberculosis clinic as an objective method for decision as to activity and as a guide in the treatment to be employed.

DEXTROSE, Normal Renal Threshold for, Campbell, R. A., Osgood, E. E., and Haskins, H. D. Arch. Int. Med. **50**: 952, 1932.

The authors studied 34 persons, 22 of whom gave satisfactory results. Their conclusions were that filtrates obtained by Somogyi's zinc sulphate-sodium hydroxide method of precipitating blood proteins give true sugar values within a range of 7 mg. per hundred cubic centimeters.

Filtrates obtained by the tungstate precipitation method give results within a narrow range, averaging 21 mg. higher than the true sugar; hence, tungstate methods are clinically satisfactory, and this factor, 21 mg., can be used to transpose results from one method to the other.

It seems to be impossible to produce an alimentary glycosuria in some normal persons by the ingestion of large amounts of dextrose.

The renal threshold for true sugar varies from 99 to 228 mg. per hundred cubic centimeters, 80 per cent of cases having values that range from 140 to 190 mg.

If a normal renal threshold is to be given, it must allow for wide variation. The authors suggest, from the probable error of 15.5 mg. and the mean of 151 mg. of this series, a probable range of from 105 to 200 mg. for true sugar, or from 125 to 220 mg. if the tungstate precipitation method is used.

BLOOD, Macroscopic Examination of, Wintrobe, M. M. Am. J. M. Sc. **185:** 58, 1933.

The following procedures are advocated and described:

By a series of consecutive steps which are simple and may be quickly performed, it is possible by the use of a single instrument, a modified hematocrit, to gain accurate and valuable information concerning the blood.

1. Five cubic centimeters of blood are drawn from a vein with a dry syringe and needle and placed in a small bottle containing 10 mg. of potassium oxalate with which the blood is thoroughly mixed.

2. The hematocrit is filled to the "10" mark by means of a capillary pipette and sedimentation rate is determined. The sedimentation rate serves as a guide to the presence and intensity of organic destructive or infectious disease.

3. The filled hematocrit is next centrifugalized until no further packing of the corpuscles takes place. The volume of packed red corpuscles, and the volume of packed leucocytes and platelets may then be read.

The volume of packed red cells is an accurate index of the degree of anemia and is more easily and more accurately measured than even of the red cell count. It also serves to correct the sedimentation rate of the blood, as above determined, for the influence of anemia or polycythemia. Furthermore, if the red cell count and hemoglobin value of the sample of blood collected for hematocrit studies has been determined, the mean volume and hemoglobin content of the red corpuscles may be calculated. The latter information is of great value not only in differentiating the anemias but also in serving as a guide to the type of treatment which may be expected to be effective.

The volume of packed white cells and platelets serves as a rough guide to the quantity of these corpuscles in the blood.

4. Finally, the icterus index of the plasma may be read and this, in the absence of liver disease, biliary obstruction and carotinemia, serves as a useful guide to the degree of blood destruction.

DEXTROSE TOLERANCE, Decreased in Acute Infectious Diseases, Williams, J. L., and Dick, G. F. Arch. Int. Med. **50:** 801, 1932.

The series studied consisted of one hundred and eight patients, including sixty-seven patients with scarlet fever, seventeen with diphtheria, eight with pneumonia, five with influenza, three with acute tonsillitis, three with measles and one each with erysipelas, encephalitis, mumps, epidemic meningitis and poliomyelitis. Ten normal subjects are also included.

The authors state that glycosuria occurred in 41 per cent of patients with acute infectious diseases. The largest average amount of dextrose was excreted by the patients with influenza and miscellaneous acute infections.

This glycosuria is accompanied by a lower carbohydrate tolerance, as shown by dextrose tolerance tests and blood sugar curves both in acute infectious diseases and in experimental infections in animals.

Administration of insulin improves the dextrose tolerance in acute infections.

This work suggests that in infectious diseases there is often an injury to the islets of Langerhans with a lessened production of insulin.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Streptococci in Relation to Man in Health and Disease*

THE student of modern bacteriology, indeed, even the casual observer of the variety and multiplicity of its recent advances must perforce feel that many of its later developments have primarily emphasized the inherent vastness and complexity of the subject.

Particularly may this be said to be true in the study of that exceedingly extensive and important group, the streptococci, the importance of which as related to the production of disease and the complexity of which as concerns their species specific activities and differentiation is only recently becoming fully appreciated.

Initiated, perhaps, by the outstanding contributions of Brown in the establishment of the basic classification now generally accepted, the literature upon the streptococci has assumed mammoth proportions culminating in the exceedingly comprehensive work of the Thomsons working in the Pickett-Thomson Laboratories.

To correlate all this work and to present it in a succinct yet comprehensive fashion is the purpose of Dr. Williams' book.

That some such attempt has been greatly needed will readily be granted. That it has been done by one so well fitted by experience and extensive personal investigation in this field assures the reader of a well-planned and equally well-executed volume practically indispensable to the library of the bacteriologist as well, indeed, to all interested in the study of disease.

The first three chapters present a general resume of the subject and include a historical survey and classification; a discussion of general characteristics and their utility in the differentiation of species; and the incidence and general effect of these organisms on man.

Subsequent chapters are devoted to the relation of streptococci to local infections; to general infections; to streptococci in erysipelas; in scarlet fever; in septic sore throat; to a discussion and tentative classification of Beta hemolytic types; to streptococci in rheumatism, arthritis, and allied conditions; in diseases classed as virus diseases; and, finally, to general considerations.

The bibliography of twenty-seven pages is appended.

The format and general make-up of the book are in keeping with the productions of this publisher. Typographical errors are exceptionally few.

There can be no doubt that this volume is a contribution of the first importance; that it will be received with acclaim by bacteriologists in general, and that it will remain as a standard reference for some time to come.

It can be recommended without reserve.

The Sputum—Its Examination and Clinical Significance†

IT IS quite true, as the author states, that with exception of certain routine studies such as search for tubercle bacilli, examination of the sputum is becoming to a certain extent a lost art, with a consequent failure to obtain information that is of paramount diagnostic significance in certain cases.

The author in this monographic volume has compiled and correlated various observations on examination of the sputum so that they are readily available in compact form. It is interesting however after careful perusal of the volume to note that little of new has been added to our knowledge of the sputum in the recent years.

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*Streptococci in Relation to Man in Health and Disease. By A. W. Williams, M.D., First Assistant Director, Bureau of Laboratories, Department of Health, New York. Cloth, 260 pages, 7 plates. Williams & Wilkins Co., Baltimore, Md.

†The Sputum. Its Examination and Clinical Significance. By Randall Clifford, M.D., Associate in Medicine, Peter Bent Brigham Hospital; Assistant in Medicine, Harvard Medical School; Formerly Associate Physician and Director of Pulmonary Clinic, Massachusetts General Hospital. Cloth, 167 pages. The Macmillan Company, New York, 1932.

The author's microscopic routine consists in the preparation of four slides, one unstained, another stained for tubercle bacilli, the third with Smith's Gram-Eosin Stain and the fourth with the Fontana Stain for Vincent's organisms. He very properly emphasizes the importance of search for parasitic fungi.

Clinical Atlas of Blood Diseases*

THE book, arranged as a convenient small handbook, contains a series of most excellent colored plates representing the blood smear in various diseases. The first eight plates depict the various blood elements, erythrocytic and leucocytic, normal and abnormal, which may appear in the circulating blood, arranged in the order of their developmental sequence. Plate 1 depicts the development of granular leucocytes, Plate 2 of lymphocytes, Plate 3 of the erythrocytes. Plate 4 depicts the varieties of myeloblasts and premyelocytes; Plate 5 of the granular leucocytes; Plate 6 megakaryocytes and normoblasts; Plate 7 other abnormal cellular elements of the blood; and Plate 8 types of neutrophilic granular leucocytes. The remaining thirty plates depict typical pictures in the various blood diseases.

For reference value both in teaching and in diagnostic work this compact volume will find a very useful place.

Anatomy and Physiology**

BUNDY'S Textbook of Anatomy and Physiology for Nurses, edited by Dr. Weeder, is now in its sixth edition. Its arrangement follows very much that of preceding editions and the volume can be well recommended for the use of nurses and others who wish to acquire a good, general knowledge of the structure of the human body and its functions. Anatomy and physiology are correlated throughout the book rather than being kept separate and distinct.

Biochemistry in Internal Medicine†

A COMPACT volume on normal and abnormal physiology as it can be studied clinically by biochemical methods. Of value as a students' textbook and a reference manual. Subject matter includes carbohydrate, protein and cholesterol metabolism, the metabolism of chloride, calcium and phosphate, acid-base balance, respiratory exchange, diabetes, renal function, hepatic function, pancreatic function and kindred subjects. The last chapter contains a concise outline of clinical chemical changes in various disorders, indicating the nature of studies that should be made in each of the diseases and the findings usually observed.

Man and Microbes‡

A MOST enjoyable written primer for the laity on the history of bacteriology and its application in agriculture, industry, public health, and medicine. Abundantly illustrated and written by an authority on the subject. Not too long.

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*Clinical Atlas of Blood Diseases. By A. Piney, M.D., M.R.C.P., Director of Pathological Department, The Cancer Hospital, London, Consulting Pathologist, Chelmsford Hospital and Stanley Wyard, M.D., M.R.C.P., Physician, The Cancer Hospital, London, and Princess Beatrice Hospital. Second Edition. With 38 illustrations, 34 in color, cloth, pages 105. P. Blakiston's Son and Company, Inc., Philadelphia, 1932.

**Textbook of Anatomy and Physiology for Training Schools and other educational institutions, by Elizabeth R. Bundy, M.D., Formerly Adjunct to the Professor of Anatomy in the Woman's Medical College of Pennsylvania and Superintendent of Connecticut Training School for Nurses, New Haven, etc. Sixth Edition, Revised and Enlarged by S. Dana Weeder, M.D., Instructor in Anatomy, University of Pennsylvania. Cloth, 446 pages. P. Blakiston's Son and Company, Inc., Philadelphia, 1930.

†Biochemistry in Internal Medicine. By Max Trumper, Ph.D., Clinical Chemist and Toxicologist; formerly in charge of the Laboratories of Biochemistry of the Jefferson Medical College and Hospital and of the Psycho-Biochemistry Laboratory, Graduate School of the University of Pennsylvania, and Abraham Cantarow, M.D., Instructor in Medicine, Jefferson Medical College; Assistant Attending Physician, Philadelphia General Hospital; in charge of Laboratory of Biochemistry, Jefferson Hospital. With a foreword by Elmer H. Funk, M.D., Sutherland M. Prevost, Professor of Therapeutics at Jefferson Medical College. Cloth, pages 454, with illustrations. W. B. Saunders Company, Philadelphia and London, 1932.

‡Man and Microbes. By Stanhope Bayne-Jones, M.D. Professor of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y. Cloth, pages 128. Illustrated. The Williams and Wilkins Company and Associates in Cooperation with The Century of Progress Exposition, Baltimore, 1932.

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EDITORIAL

Suprarenal Hemorrhage in the Newborn

ALTHOUGH at any age the adrenal glands are essential to life, there is evidence that they subserve a special function during the antenatal period. In the fetus the adrenal, like the liver, is proportionately very much larger than after birth. At birth it is about one-third the size of the kidney, while in the adult it is about one-thirtieth of the size of the kidney. While the individual growing to adult size increases in weight about twenty times, the adrenal glands increase only to about double their original weight. The antenatal adrenal is especially rich in cortical tissue, the secretion of which as we shall see appears to serve an especially important rôle prior to birth.

Immediately after birth pronounced structural changes become manifest in the organ. The inner or central cortical layers disintegrate and are replaced with vascularized connective tissue. Only a very small portion of the cortex immediately beneath the capsule escapes this degenerative process. The medulla appears to grow into the new connective tissue frame work, replacing the former inner layer of the original cortex.

These changes are clearly recognizable in the first two weeks of life. They are slow however and do not reach their greatest stage until about the end of the first year. Regeneration of cortical material commences in the second year and according to Goldzieher and Gordon does not reach full development until adolescence.

These authors in a review of the subject conclude that in all probability the cortical hyperplasia in fetal life is connected with the respiratory metabolic needs of the fetus. They believe that large amounts of cortical material are necessary during fetal life to maintain tissue respiration. After birth, with the onset of respiration and the increase of oxygen tension in the blood, lesser amounts of cortical hormone are required to maintain tissue respiration and the cortex therefore involutes to some extent. The oxygen tension in the blood is higher after birth than prior thereto. Comparable observations have been made in cyanid poisoning and beri-beri. In both conditions tissue respiration is interfered with and there results a compensatory hypertrophy of the adrenal cortex. Apparently the adrenals attempt to produce more cortical hormone, to overcome the impaired tissue respiration in chronic cyanid poisoning.

The suprarenal gland is one of the most vascular tissues if not the most vascular in the body. Each minute six times its weight of blood passes through the organ. It is not surprising therefore that occasionally during the involutionary process of this extremely vascular organ hemorrhages occur which may become sufficiently extensive to be productive of symptoms. Massive suprarenal hemorrhage in the newborn was first described in 1837 and it has been recorded many times since. At first it was described only as a postmortem curiosity and for a long time it has been felt that the symptomatology of adrenal hemorrhage was so obscure that there was little probability of establishing a symptom complex which would facilitate ante mortem diagnosis and treatment. In recent years, however, attempts have been made to do this, often with success. Two of the more recent contributions on this subject, those by Goldzieher and Gordon and by Arnold have served to emphasize the importance of the disease, the possibility of its clinical recognition, and the curative therapeutic possibilities.

The phenomenon is especially apt to manifest itself in the first few days of life, following the same time range as that of hemorrhage of the newborn, and may be but a part of the picture of this condition; or it may occur following birth trauma, asphyxia, eclampsia, or as a complication of syphilis, hemophilia, extensive burns, diphtheria or embryonic cystic processes. It does occur occasionally at later ages. Goldzieher and Gordon were able to collect reports of 37 cases of adrenal hemorrhage in the newborn and 38 in older persons, in which clinical information was available and to these they added 6 additional cases 5 of which were diagnosed during life, making a total of 81 cases. The lesions varied from small hemorrhages, through diffuse hemorrhagic infarction, to destructive hemorrhages, and massive hemorrhage which occasionally ruptures into the peritoneal cavity.

The important points in the symptomatology as outlined by these authors are high fever, hemorrhagic rash, rapid respiration, cyanosis, convulsions and evidences of internal hemorrhage.

The fever may be a part of a general infection in which adrenal hemorrhage appears as a complication or it may occur in the absence of infection. The explanation offered is that the adrenal medulla participates in the regulation of body tem-

perature by stimulating the sympathetic nervous system, and particularly the thyroid gland, while the cortex checks the heat producing activity of the thyroid. It has been shown that destruction of a sufficiently large proportion of cortical tissue produces pyrexia, provided thyroid function is unimpaired. These authors as well as Arnold however point out that the temperature may remain normal, even in massive hemorrhage, presumably when there has not been too great cortical destruction.

Rapid respiration is sometimes so pronounced a symptom as to give rise to an erroneous diagnosis of pneumonia. The term "pseudo pneumonia of the newborn" has been suggested as descriptive of the phenomenon. Bilateral adrenalectomy in dogs results in rapid respiration and hyperventilation of the lungs. The administration of cortical hormone restores normal respiratory rate. Goldzieher injected cortical hormone in a case of adrenal hemorrhage, reducing the rate from 50 to 31 per minute. The function of the cortical secretion in controlling tissue respiration has been discussed above and presumably the rapid respiration observed in some cases of adrenal hemorrhage is a result of cortical destruction. This symptom is said to be more pronounced in the new born than in older children or adults with adrenal hemorrhage.

Hypoglycemia also occurs, probably due to medullary insufficiency.

Arnold emphasizes the diagnostic importance of the symptoms of internal hemorrhage with collapse, comparable to those observed in ruptured ectopic pregnancy, often with a palpable abdominal tumor in the region of the kidneys, pronounced abdominal distention, sometimes with a bluish discoloration, sometimes a doughy consistency to the abdomen due to intraperitoneal blood, and often pain which may be excruciating. Not infrequently, particularly in massive hemorrhage, the symptoms of shock may be so pronounced as to entirely mask the previously enumerated symptoms referable to endocrine disturbance, as pyrexia and rapid respiration.

Treatment is directed against (a) the internal hemorrhage, (b) the adrenal insufficiency and (c) the hypoglycemia. It has been suggested that the convulsions sometimes observed may be hypoglycemic in origin. As has been shown by Arnold and others prompt transfusion may sometimes be life saving. In those cases in which the hemorrhage is a part of the picture of the hemorrhagic diathesis of the newborn transfusion may be looked upon as curative as well as palliative. The administration of cortical hormone appears logical and has been employed in one case by Goldzieher and Greenwald with beneficial results. Glucose is, of course, indicated to combat the hypoglycemia. While death usually occurs early the illness may last for several days. One patient succumbed on the fortieth day of the illness.

Recognition of the fact that during its involutionary phase directly after birth the adrenal gland is especially subject to hemorrhagic incidents or catastrophes, and knowledge of a fairly clear cut symptom complex should eventuate in more frequent and earlier clinical recognition of suprarenal hemorrhage, with consequent increased incidence of recovery. —*W. T. V.*

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